Committee 2

Cell Biology

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ABMA	α,β-methylene ATP
ACE	angiotesin converting enzyme
ACh	acetylcholine
α-, β-actin	isoforms of actin
AITC	allyl-isothiocyanate
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate
3-APMPA	3-aminopropyl(methyl)phosphinic acid
3-APPA	3-aminopropylphosphinic acid
A—receptor	adenosine- receptor
α-receptor	alpha adrenoreceptor
AT	angiotensin
ATP	adenosine triphosphate
BOO	bladder outlet obstruction
B-receptor	bradykinin receptor
β-receptor	beta adrenoreceptor
BK_{Ca}	large conductance K^+ channel
BSMC	bladder-derived smooth muscle cell
C	bladder compliance
Ca^{2+}	calcium ion
CA	*trans*-cinnamaldehyde
cAMP	cyclic adenosine monophosphate
CaM kinase	Ca-mitogen kinase
cGK	cGMP-dependent protein kinase
cGMP	cyclic guanosine monophosphate
CICR	Ca^{2+}-induced Ca^{2+} release
c-kit (*kit*)	a protein-tyrosine kinase receptor
Cl^-	chloride ion
CNS	central nervous system
CO	carbon monoxide
Cx	gap junction protein, connexin
CPI-17	an MLCP inhibitor
DAG	diacylglycerol
4-DAMP	N-2-chloroethyl-4-piperidinyl diphenylacetate
E	elastic (Young's) modulus
EAG	ether-à-go-go-related gene
ENaC	epithelial Na^+ channel
eNOS	endothelial nitric oxide synthase
EP receptor	prostaglandin E_2 (PGE_2) receptor
ER	endoplasmic reticulum
ET	endothelin
G-protein	guanosine phosphate binding protein
GABA	γ-amino butyric acid
G-I tract	gastrointestinal tract
GRK	G-protein coupled receptor kinase
HA-1077	a rho-kinase inhibitor
HERG	human ether-a-go-go related gene
5-HT	5-hydroxytryptamine
ICC	interstitial cell of Cajal
IC-MY	ICC in longitudinal muscle layer
IC-SM	ICC in circular muscle layer
iNOS	inducible nitric oxide synthase
IL-6	interleukin-6
IMI	inter-micturition interval
IP_3	inositol trisphosphate
IPSS	international prostate symptom score
k	stiffness
K^+	potassium ion
K_{ATP}	intracellular ATP-gated K^+ channel
KCl	potassium chloride
KCNQ	voltage-gated K^+ channel, KQT-like superfamily
L_0	optimal resting length for a muscle
L2	second lumbar spinal level
LUT	lower urinary tract
m receptor	muscarinic receptor (gene level)
M receptor	muscarinic receptor (protein level)
mGluR	metabotropic glutamate receptor
MLCK	myosin light chain kinase
MLCP	myosin light chain phosphatase
MPEP	6-methyl-2-(phenylethynyl)pyridine
mRNA	messenger ribose nucleic acid
MS	mechanosensitive
mtNOS	mitochondrial nitric oxide synthase
Na^+	sodium ion
NA	noradrenaline
NANC	non-adrenergic, non-cholinergic
$NiCl_2$	nickel chloride
NIH	National Institutes of Health
NK receptor	neurokinin receptor
NMDA	*n*-methyl-D-aspartate
NO	nitric oxide
NOS	nitric oxide synthase
nNOS	neuronal nitric oxide synthase
OAB	overactive bladder
P	pressure
P2-receptor	receptors to ATP, subtype X/Y
pA_2	negative logarithm of dissociation constant
PACAP	pituitary adenylate cyclase activating peptide
4-PBA	4-phenylbutyrate
PDE	phosphdiesterase
PE	phenylephrine
PG	prostaglandin
PKA	protein kinase-A
PLC	phospholipase-C
PT	pressure threshold
RAR	recto-anal reflex
ROK/ROCK	rho-associated kinase
RT-PCR	reverse transcriptase polymerase chain reaction
S1	first sacral spinal level
SCI	spinal cord injury
SCT	spinal cord transection (transected)
SERCA	sarcoplasmic reticulum Ca^{2+}-pump
sGC	soluble guanylate cyclase
SHR	spontaneous hypertensive rat
SIS	small intestine submucosa
slo-gene	encoding the BK channel α-subunit
SK_{Ca}	small conductance K^+ channel
SM	smooth muscle myosin
SMPP-1M	a smooth muscle myosin phosphatase
SUI	stress urinary incontinence
T	wall tension
T10	tenth thoracic spinal level
7TM receptor	7-transmembrane helix receptor
TMB-8	an IP_3 receptor blocker
TREK channel	TWIK-related K^+ channel
TRPA	transient receptor potential, ankyrin
TRPM	transient receptor potential, melastatin
TRPML	transient receptor potential, mucolipin
TRPP	transient receptor potential, polycystin
TRPV	transient receptor potential, vanilloid
TTFA	thenoyltrifluoroacetone
TTX	tetrodotoxin
TUNEL	terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling
TX	thromboxane
UP	uroplakin
UPEC	uropathogenic *Escherichia coli*
UPR	unfolded protein response
UTP	uridine triphosphate
VEGF	vascular endothelial growth factor
VIP	vasoactive intestinal peptide
Y-27632	a rho-kinase inhibitor

Throughout SI (*Systeme Internationale*) units have been used, in particular based on units of length, metre (m); mass, kilogramme (kg); time, second (s); electric current, ampere (A); amount of substance (mol, M): prefixes are k (10^3), m (10^{-3}), μ (10^{-6}), n (10^{-9}). Derived units are combinations of SI units and include those for: voltage (V, = kg m^2 s^{-3} A^{-1}); resistance ($\Omega = V.A^{-1}$); conductance ($S = \Omega^{-1}$); force (Newton, N = kg.m.s^{-2}); frequency (Hz, s^{-1}). Some non-SI units, derived from SI units include gram (g), minute (min), hour (hr). Some non-SI units without a precise definition are used on occasion: these include litre (*l*, approximating to dm^3) and cm.H_2O as a unit of pressure. The molar unit of concentration (moles per dm^3 solvent) is used throughout and is donated by the letter *M*. Thus the non-standard form of concentration mol/L is avoided, as it has no meaning in the SI system of units.

Symbols for ions in solution, eg Ca^{2+}, Na^+, etc, refer to the species that are presumed to take part in chemical reactions. No assumptions are made about the activity coefficient of the species in solution. Symbols for metals, Ca, Na, etc, refer to chemical moieties and this makes no statement as to the sub-fraction that will take part in a biological process, eg Na-pump.

CONTENTS

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I. INTRODUCTION

This aim of this report is to describe the cell and tissue physiology and biochemistry of the lower urinary tract that will help to understand the pathophysiology of the overactive bladder and lower gastrointestinal (G-I) tract and to highlight potential avenues whereby the conditions may be better managed. The report will attempt to explain the most important features of the lower urinary and G-I tracts, and describe important changes that are associated with functional disorders. It is not always possible to differentiate between primary and secondary changes to tissue that are associated with tract dysfunction but this is not necessarily important if potentially useful targeted models are identified that may be used to develop new therapeutic and other approaches to manage urinary and faecal incontinence.

Since the previous consultation [1] there has been considerable advance in our understanding of the lower urinary and G-I tract, particularly with respect to the bladder. In particular the urothelium has emerged as an important tissue in mediating lower urinary tract sensations, and the principles learnt may in the future be applied to the G-I tract. Equally, our understanding of the mode of action of some useful drugs and a greater understanding of their molecular targets has been radically altered, which should enable the development of better-targeted drugs. **Figure 1** shows a diagrammatic representation of the bladder wall with some of the structures and receptors that will be described in the following sections.

Increased understanding of these tissues has also facilitated tissue-engineering approaches to develop functional implants and recent advances will be described. This report has attempted to reflect these advances. It will refer to the previous report when basic principles were described, and when some aspects were discussed in depth and re-examination might be repetitious. All referenced material is from peer-reviewed publications, most of which have appeared after the previous consultation [1].

II. THE UROTHELIUM AND SUBUROTHELIUM

1. INTRODUCTION

The previous report [1] concentrated on the structure

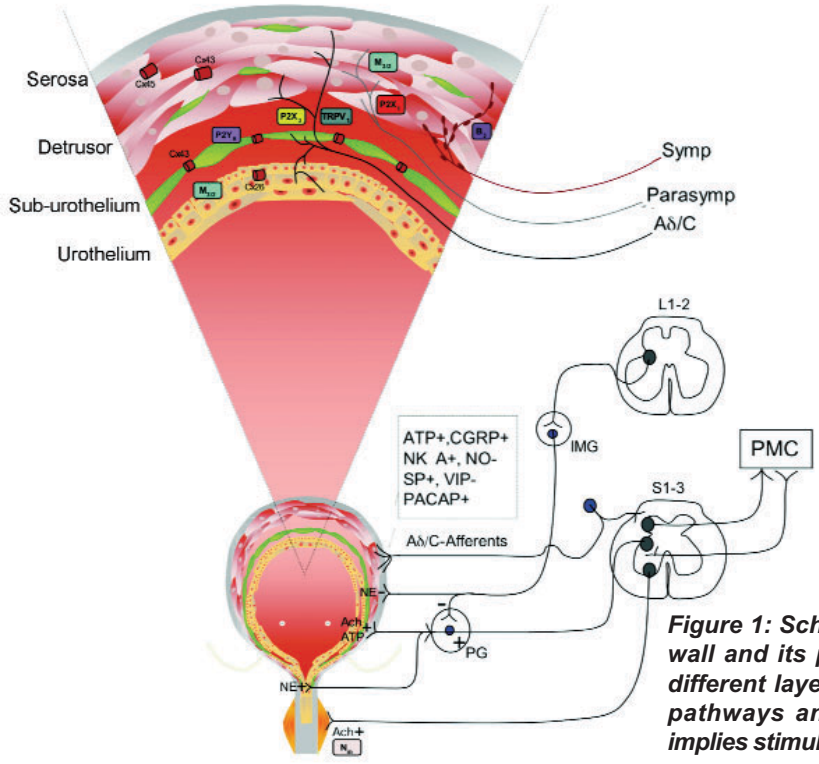

Figure 1: Schematic representation of the bladder wall and its principal nervous connections. The different layers are shown with some of the main pathways and receptors denoted. Positive (+) implies stimulatory and negative (-) implies inhibitory

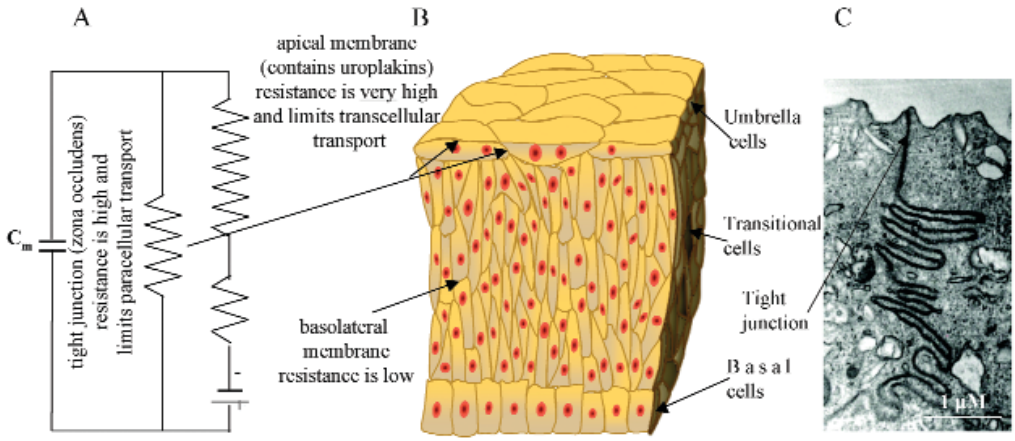

Figure 2 : The urothelium. A: An equivalent circuit diagram of the urothelium, showing the major resistances to charge (including ions) flow – the paracellular route through tight junctions, and transmembrane route. The resistances are sufficiently large that a transepithelial potential is developed, with charge separated by an equivalent capacitance, C_m. B: the major cell layers. C: electron micrograph of two adjacent umbrella cells, showing the tight junction between them. Adapted from Kanai et al. Am J Physiol 2004; 286: H13-H26.

as well as the transport and barrier functions of the urothelium, and those factors that may alter the permeability of the membrane. In the intervening period the urothelium has been increasingly recognised also to have secretory functions that allow it to behave as a sensory and signaling structure, influencing the activity of both nerves and also underlying tissue layers. In this mode it interacts closely with the underlying suburothelial layer so that the whole structure can be regarded as a functional unit. **Figure 2** shows the structure of the urothelium. Below the basal cells of the urothelium is a connective tissue layer, sometimes referred to as the lamina propria, in which is embedded a rich network of capillaries, unmyelinated and myelinated nerves and a functional syncitium of interstitial cells (myofibroblasts).

2. THE UROTHELIUM: CHANGES IN DISEASE AND BACTERIAL INFECTIONS

The urothelium consists of three layers: i) an apical (umbrella) cell layer with a very low permeability to urine and pathogens; ii) an intermediate layer; and iii) a basal layer that interacts with the extracellular matrix of the suburothelial region for structural support. The barrier is determined by: the low mobility of the aliphatic chains in phospholipid molecules (high degree of saturation, high concentrations of sphingomyelin and cholesterol) [2]; segregation of low mobility lipids into the outer leaflet of the apical membranes [3]; the presence of uroplakin proteins in the apical membrane and the presence of tight junctions between adjacent cells [4,5]. When the bladder is empty, large numbers of vesicles underlie the apical membrane. As the bladder fills, these vesicles are inserted into the apical membrane to maintain a constant relationship between the area of apical membrane and the volume of the bladder. The role of uroplakins and defects in their structure in relation to lower urinary tract anomalies remains unclear. Ablation of the uroplakin-II gene in mice [6] or loss of uroplakin expression in patients with myelomeningocele [7] led to hyperplastic growth of the urothelium and may interfere with the development of underlying smooth muscle. In addition, UPIII expression is a powerful prognostic factor in patients with upper urinary tract urothelial carcinoma [8].

Uropathogenic *Escherichia coli* (UPEC) is the main causative agent for urinary tract infections in women [9]. With animal models of cystitis UPEC have part of their pathogenic cycle as an intracellular phase within urothelial cells where replication and formation of bacterial communities occurs [10], before exiting the host cell. Invasion is facilitated by adhesive fibres - type-I pili [11]. Thus, UPEC can form hidden intracellular reservoirs of bacteria that can persist for several weeks that may be protected from antibiotics. Whilst much of the formative work has been carried out on animal models, recently intracellular bacterial communities from exfoliated urothelial cells have been detected in the urine of patients [12].

3. SECRETORY AND SIGNALING PROPERTIES OF THE UROTHELIUM/SUBUROTHELIUM

In recent years the ability of the urothelium to respond to stimuli such as stretch and osmolarity changes, and its release of various chemical factors including substance P [13], nitric oxide (NO) [14], ATP [15] and ACh [16], has been recognised. Histological studies have shown there are many sensory neurons located in the urothelial/suburothelial region that label for receptors to these factors, or contain sensory peptides. Moreover, the extent of labeling is altered in conditions that result in bladder overactivity, or in the presence of agents designed to attenuate the condition [17-20]. The proximity of these afferent nerves therefore implies that they could interact with the urothelium to detect changes in bladder fullness. Urothelial cells themselves also express sensory receptors typically found on primary afferent nerves; including P2X/Y-receptors [21,22], $TRPV_{1,2,4}$ [23,24], $TRPA_1$ [25], $TRPM_8$ [26], $B_{1,2}$ bradykinin receptors [27], adrenergic

receptors [14,28], nerve growth factor receptors [29] and amiloride-sensitive Na^+ channels [30-32].

One of the first stretch-released factors to be identified in the urothelium was ATP, which was released in response to stretch or hypotonic stimulation from various species [15] including humans [33]. **Figure 3** shows that stretch-dependent ATP release from the bladder wall is dependent on an intact mucosa, and that release is attenuated by amiloride. The latter observation supports the hypothesis that epithelial Na^+ channels regulate ATP release (see section VIII 2-b). It is hypothesized that purinergic receptors on sensory neurons and/or myofibroblasts are targets. $P2X_3$ receptors have been identified as the likely target for activation of suburothelial afferents. This suggests that $P2X_3$ receptors are involved in sensory activation during the filling phase, as inferred from observations of $P2X_3$ knockout mice, which exhibited a reduced afferent firing and micturition reflex [34,35]. The relevance of this mechanism is illustrated in **figure 4**, where it is observed that intravesical amiloride increases inter-micturition interval [446].

The intrinsic activity of suburothelial myofibroblasts was potentiated by P2Y receptor activation [36], most likely through the $P2Y_6$ subtype [37]. This suggests that there are multiple targets for urothelial-derived ATP. The release of ATP increases when damage occurs to the urothelial layer, for example, in interstitial cystitis [38] or spinal cord injury [39] and may be reduced by treatment with botulinum toxin, for example [40]. There is also a marked increase in the expression of the gap junction protein connexin26 in the urothelium [41]. The enhancement of cell-cell communication may lead to increased sensitivity and propagation of signals through the urothelium in response to stimuli. Therefore, it may be hypothesized that increased urothelial ATP is a contributing factor to afferent sensitization through enhanced activation of suburothelial nerves and/or myofibroblasts.

ACh also is released from the urothelium in response to stretch; the amount increases with age and oestrogen status [42-44]. Most likely this release is through a non-vesicular mechanism, mediated by organic cation transporter type-3 [45]. Urothelial-derived ACh, like ATP, may also have a role in promoting sensory activation. This arises from the fact that anticholinergic drugs reduce detrusor overactivity and urge during the filling phase of the bladder, when efferent nerves are not activated [46].

Hence, it can be hypothesized that anticholinergics are not acting on the muscle but elsewhere, possibly muscarinic receptors in the urothelium. Recent studies investigated the localization of muscarinic and nicotinic receptors in the human [47] and mouse [48] urothelium. All five muscarinic subtypes were expressed throughout the urothelial layers. There was specific localization of the M_2-subtype to the umbrella cells and M_1 to the basal layer, and M_3 receptors more generally distributed. M_3, but not M_2, receptor expression was reduced in human tissue from patients with idiopathic detrusor overactivity. However, it is not known if this was evident throughout the urothelium/suburothelium or confined to a specific region [49]. The mechanism by which ACh modulates these activities is still unclear. However, blockade of urothelial muscarinic receptors with atropine inhibits stretch-induced ATP release [50]. Stretch-released ACh may therefore act in a feedback mechanism to induce basolateral ATP release. Thus, the urothelium is clearly more than a barrier, demonstrating a role in modulating bladder contractile and sensory activities. The cellular transduction mechanism from the urothelium is still unclear, but elucidation of how the urothelium communicates with the detrusor and the sensory nerves could uncover potential therapeutic targets.

A network of interstitial cell-like myofibroblasts have been identified in the suburothelium that label for vimentin, are connected by gap junctions containing Cx43 (**Figure 5**) and are closely apposed to unmyelinated nerve endings [51,52] and label with *c-kit* [53]. Isolated myofibroblasts respond to exogenous ATP (*via* P2Y receptors) by generating nifedipine-resistant intracellular Ca^{2+} transients and a subsequent Ca^{2+}-activated Cl- current [36,54]. At the prevailing negative resting potential, this current generates a transient depolarization. These responses mirror spontaneous Ca^{2+} transients and inward current [36].

Thus, local release of ATP from the urothelium may be postulated to generate depolarizing Ca^{2+} waves that spread across the myofibroblasts network and thus amplify and modulate local responses to endogenous ATP. The ability of myofibroblasts to form functional networks can involve mechanisms other than via gap junctions: if two isolated cells are pushed together, each cell demonstrates enhanced responses to ATP without the obvious formation of gap junctions. Cadherin-11 has been demonstrated on myofibroblast membranes [55] and their activation by intercellular adhesion may offer a mechanism. This enhancement of response is abolished by the *c-kit* receptor tyrosine kinase inhibitor, glivec.

These purinergic responses are mimicked by extracellular acidosis and attenuated by capsaicin and NO donors. The latter effect is in keeping with the demonstration of NOS/guanylate cyclase activity in these cells [56]. The effect of extracellular acidosis and capsaicin is of significance as it proposes a mechanism whereby the bladder wall may respond to local ischemia – a feature of bladder filling – especially in the presence of outflow obstruction or reduced compliance [57-59].

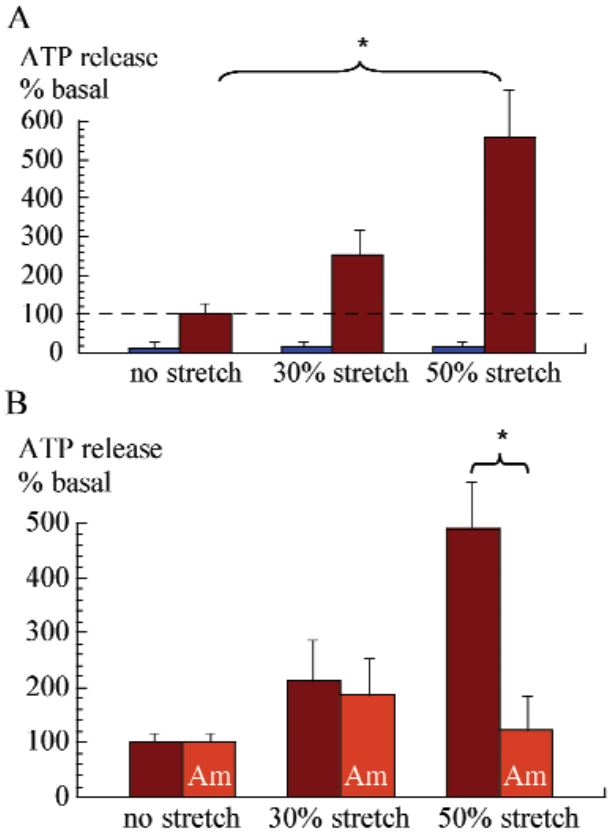

*Figure 3 : ATP release from the bladder during lateral stretch. A: ATP release from a section of the bladder wall, with the mucosa removed (left-bar of pair) or retained (right-bar of pair). Wall segments were held at slack length, or stretched by 30 and 50% of the slack length. Values normalised to release at slack length with mucosa, *p<0.05. B: The effect of 1 mM amiloride on ATP release from the bladder wall – mucosa intact – under different degrees of stretch, *p<0.01.*

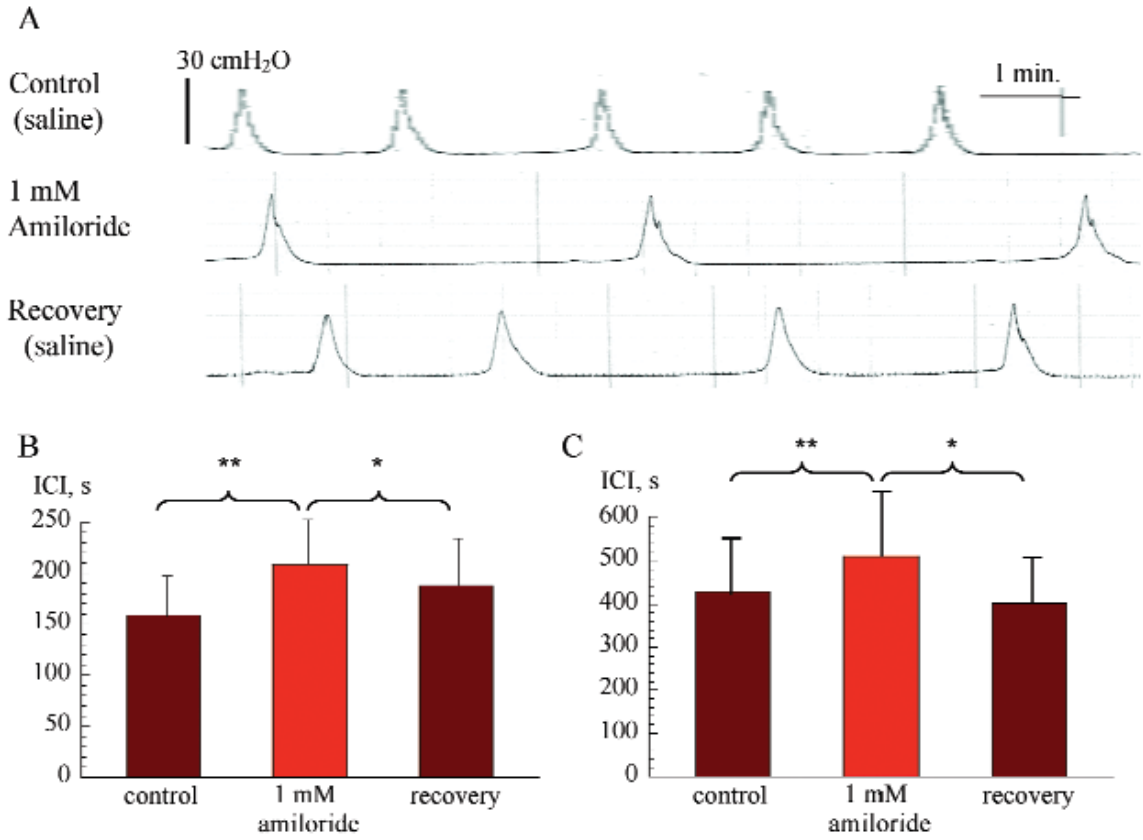

*Figure 4 : The effect of intravesical amiloride on the micturition reflex in urethane-anesthetized rats. A: typical tracings of continuous cystometry (CMG) during filling: before, during and after washout of amiloride. B: the effect of amiloride on intercontraction interval (ICI) in normal rats. C: the effect of amiloride on intercontraction interval (ICI) in rats with outflow tract obstruction, * p=0.01; ** p<0.01. From [446]*

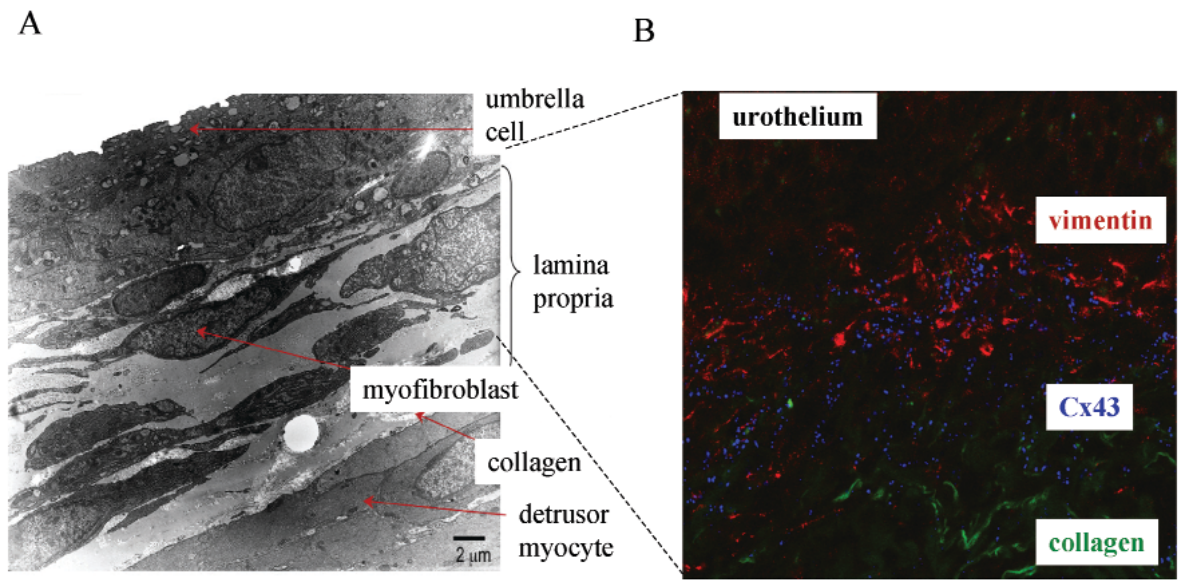

Figure 5 : The urothelium-suburothelium. A: electron-micrograph of a section, with the region referred to as the lamina propria shown. B: micrograph of a similar region showing labelling for vimentin, connexin43 (Cx43) and collagen. Unpublished data: AJ Kanai, CH Fry, G Sui.

Muscarinic M_2 and M_3 receptor labeling has also been localized to suburothelial myofibroblasts and is increased in samples from idiopathic overactive bladders; an increase in M_2 receptor labeling was also seen in samples from patients with painful bladder syndrome [60]. Isolated myofibroblasts do not respond to exogenous muscarinic receptor agonists by a rise of intracellular [Ca^{2+}], so the intracellular signaling mechanisms remain unknown [36].

However, these observations may be of significance as it has been demonstrated that C-fibre and Aδ-fibre afferent firing in response to bladder filling was reduced by relatively M_3-selective antimuscarinics [61], or less-selective agents such as oxybutynin [62] or tolterodine [63]. The latter study was of additional interest as it demonstrated that the decrease of afferent activity persisted after desensitization of a proportion of the afferents with resiniferatoxin.

4. INTERACTIONS BETWEEN UROTHELIUM/ SUBUROTHELIUM AND DETRUSOR

Several lines of evidence indicate that the urothelium/suburothelium directly modulates detrusor function, through inhibitory and excitatory mechanisms. Using in vitro detrusor preparations the potency and the maximum contractile response to acetylcholine, but not KCl, are reduced if the urothelium is intact. The substance is unknown at present, it is diffusible but is not NO, adenosine, GABA, a cyclo-oxygenase product or mediated by the small conductance Ca^{2+}-sensitive K^+-channel [64-67]. However, whether it involves activation of a beta-adrenoreceptor or the release of a beta-agonist is controversial [65,68].

Of interest is that a similar phenomenon is present in preparations from ureter, but in this case may involve a cyclo-oxygenase product [69].

Optical imaging of transverse sections of the bladder wall shows propagating Ca^{2+} and membrane potential waves in the suburothelial layer in response to physical stretch or very low concentrations of carbachol. After a delay these responses spread to the detrusor layer initiating activity there [70], **figure 6**. Optical imaging of bladder sheets, with the urothelial surface uppermost showed similar propagating waves elicited by UTP in the presence of a urothelium/suburothelium, but absent if it was removed [71]. UTP was chosen as this purine elicits excitatory responses from subu-rothelial myofibroblasts, but not directly from detrusor itself. Isolated strips of detrusor generate spontaneous activity, especially if the urothelium/ suburothelium is intact, and such activity is also up-regulated by exogenous UTP [71].

Thus, there is evidence that a suburothelial population of myofibroblasts is an intermediate stage in the sensory response to bladder wall stimuli. It acts as a variable amplifier of the sensory response mediating signals between the urothelium and sensory afferents or the detrusor smooth muscle layer, either directly or via the activation of afferent nerve fibres [72].

Moreover, the increase of myofibroblast numbers in conditions associated with bladder overactivity [41,73] suggests it is a mechanism that may be targeted to alleviate this condition. Agents that modulate myofibroblast activity, such as glivec, also reduce spontaneous activity in the bladder [74,75].

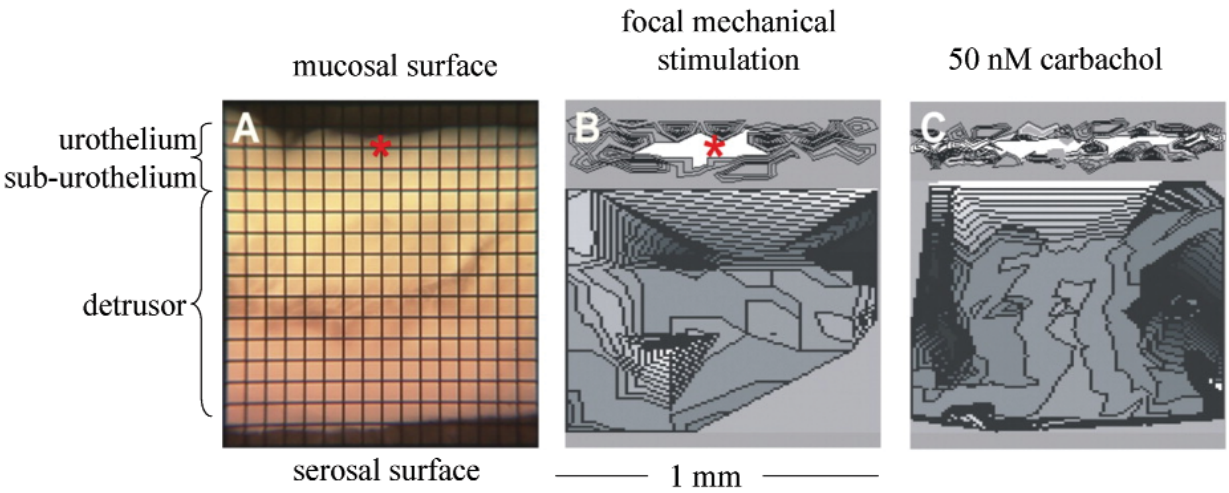

Figure 6 : Optical images through the bladder wall. A: photograph of a section through the bladder wall, with a superimposed grid. Simultaneous measurements of intracellular Ca^{2+} were made from each grid square, to record spontaneous or evoked transients. B: isochronal maps for the spread of Ca^{2+} transients after focal stimulation at the red star. The darker the shading the longer are the isochrones from the initial evoked Ca2+ transient. It may be noted that conduction initially occurs along the suburothial space. Only after a delay does the wave appear in the detrusor layer for further transmission. C: similar isochronal maps after topical application of a 50 nM carbachol solution to the apical surface. A similar pattern of suburothelial transmission, followed after a delay by propagation in the detrusor layer is observed. Figure reproduced in part and modified from [70], and used with permission.

III. CELL PHYSIOLOGY OF MUSCLE CONTRACTION: DETRUSOR

1. CONTRACTILE MECHANISMS

Smooth muscle cells of the bladder are spindle shaped single nucleated cells organized into bundles separated by connective tissue. The thin filaments are composed of α-and β-actin, that are attached to dense bodies on the cell membrane. The thin filaments provide the binding sites for the myosin thick filaments. There are four myosin isoforms that exhibit different contractile properties—smooth muscle myosin (SM) 1A, 1B, 2A and 2B. Adult bladders are composed of approximately equal amount of SM1B and SM2B (the SM1B:SM2B ratio is ~1) [76]. SM1 produces more force than SM2; SM-A types are more slowly contracting than SM-B. In obstruction, there is a shift to more SM1A, which therefore results in slower and more forceful contractions to overcome increased resistance. A detailed description of the contractile proteins and associated intermediate filaments has been recently published [77].

An increase of the sarcoplasmic [Ca^{2+}] from a basal level of 50-100 nM is required to initiate detrusor contraction, half maximal activation is achieved at about 1 μM [78]. The source of Ca^{2+} can be extracellular, via L- and T-type Ca^{2+} channels [79,80] or from intracellular stores [81]. Release from intracellular stores may be separately mediated by activation of IP_3 receptors, as it can be blocked by receptor inhibitors, or via ryanodine receptors [82]. The increase of the sarcoplasmic [Ca^{2+}] is transient and Ca2+ are either removed from the cell via Na+/Ca^{2+} exchange [83], or re-accumulated in the intracellular stores via a SERCA pump; the activity of the latter is modulated by intracellular proteins, such as phospholamban [84]. As with other smooth muscles, the contractile proteins are activated by phosphorylation of myosin by a myosin light chain kinase (MLCK), which in turn is activated by a $Ca^{2+}{}_4$-calmodulin complex. Relaxation will occur if the myosin light chain is dephosphorylated, by a myosin light chain phosphatase (MLCP), which in the pig bladder is SMPP-1M phosphatase [85]. The sensitivity of the contractile system can therefore be altered by altering the activities of MLCK or MLCP. A schematic diagram of contractile activation is shown in **figure 7**.

MLCK activity is decreased by itself being phosphorylated which cold be achieved via a number of kinases including: CaM kinase II, mitogen-activated protein (MAP) kinase, cAMP-dependent kinase (PKA) and p21-activated kinase [86,87], although details of the pathways that modulate MLCK activity in detrusor are unclear. MLCP activity can also be reduced by phosphorylation, which would increase the Ca^{2+}-sensitivity of the contractile system. Of significance is inhibition of MLCP activity by *rho*-associated kinase (ROK/ROCK) [88], which in turn is activated by small G-proteins of the *rho*-family. In detrusor the two isoforms of ROCK (I and II) have been identified [88]. Inhibitors of ROCK activity, such as Y-27632 and HA-

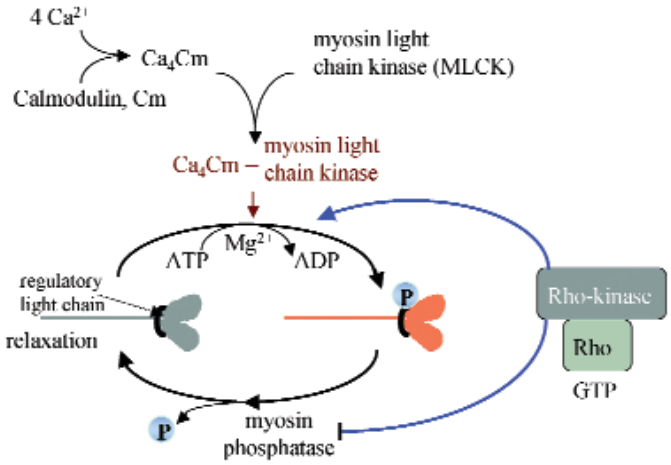

Figure 7: Schematic diagram of myosin activation (phosphorylation) and inactivation (dephosphorylation) by the calcium-calmodulin complex and other modifying agents.

1077, attenuate nerve-mediated and carbachol-activated contractions, as well as contractures in cell permeabilised preparations, but do not affect depolarization-mediated (with high [KCl]) contractures [89-92], which suggests that the *rho*-kinase pathway plays a role in the contractile state of the bladder. In addition ROCK labelling in the obstructed bladder was increased, and may contribute to the increased contractile state [92]. CPI-17, in its phosphorylated form, also inhibits MLCP [93,94], and may be so activated by protein kinase-C, as well as *rho*-kinase. Increased levels of CPI-17 in bladders of diabetic animals have been proposed as mediators of their increased contractile activity [95] – see figure 7.

2. ELECTRICAL ACTIVITY AND ION CHANNELS

Detrusor smooth muscle is an electrically excitable tissue capable of generating evoked and spontaneous action potentials [96]. The upstroke phase is carried by Ca^{2+} ions, predominantly through L-type Ca^{2+} channels, and repolarisation is mediated by K^+ efflux through several K^+ channels [79,97]. Ca^{2+} influx through Ca^{2+} channels is sufficient to elicit further release from intracellular stores [98] and sustain contractions. T-type Ca^{2+} channels have also been described in detrusor muscle and the proportion of total inward Ca^{2+} current is increased in cells from overactive bladders [99]. Because T-type channels are activated at more negative membrane potentials it was proposed that they could contribute to increased spontaneous activity in the overactive bladder [100]. Exposure of detrusor muscle strips to low $NiCl_2$ concentrations, when a selective T-type channels inhibition is achieved, indeed attenuated spontaneous contractile activity [101].

A number of receptor modulators that alter detrusor contractility affect also the L-type Ca^{2+} current. Antimuscarinic agents such as propiverine, attenuate L-type Ca^{2+} current [102-104]. The effect is probably mediated via M_3 receptors as the action is blocked by 4-DAMP [104]. β-agonists also attenuate Ca^{2+} current by a cAMP/protein kinase A-dependent mechanism [105], whilst the antispasmodic agents alverine citrate reduced action potential repolarisation rate, which was interpreted as an inhibition of Ca^{2+} current inactivation, thus increasing Ca^{2+} influx [106].

The most significant K^+ channel in detrusor is the Ca^{2+} activated large conductance K+ channel (BK_{Ca}). This channel has a physiological role in determining membrane potential, action potential repolarisation [107,108] and regulating contractile events [109,110]: channel opening is coupled to intracellular Ca^{2+} sparks emanating from ryanodine receptors [108]. Outward current is also modulated by Ca^{2+}-current influx through L-type and T-type Ca^{2+} channels. In the former case this has been proposed as a mechanism to regulate Ca^{2+} influx into the myocyte [111], and in the latter case as a basis for spontaneous fluctuations of membrane potential [100]. Reduction of BK channel activity may contribute to myogenic bladder overactivity, as deletion of the *slo*-gene that encodes for the channel protein enhances muscle sensitivity to cholinergic and purinergic agonists [112], conversely injection of *slo*-cDNA reduced overactivity [113]. BK channel activity is regulated by phosphorylation of the pore-forming α-subunit, or associated proteins [114], and affords a mechanism whereby cAMP and cGMP, through PKC, can regulate channel function [115,116]. Conversely, the Ca^{2+}-dependent phos-phatase, calcineurin, decreases BK_{Ca} conductance [117] so that overall Ca^{2+} exerts a complex control of channel function – the ability to identify molecular targets for this in channel is considered in section VIII 3-c.

Intracellular ATP-gated K^+ channels (K_{ATP}) have also been described in detrusor smooth muscle, and channel openers hyperpolarise the cell and reduce spontaneous activity. A problem with the use of these channel modulators is that of tissue specificity, as many are as potent, if not more, in generating similar responses in vascular smooth muscle. Agents with greater uro-selectivity have been developed [118] but there has been little progress with the use of such agents to attenuate bladder overactivity.

Stretch-activated channels could serve a dual purpose in the detrusor myocyte: to permit cation influx to depolarize the cells and thus cause contraction to counter the initial stretch, and to initiate intracellular signaling cascades that may initiate cellular reconfiguration or growth [119,120]. Physical stretch of detrusor myocytes opens non-selective cation channels, depolarizes the cell and augments Ca^{2+} influx through Ca^{2+} channels [121]. Stretch also increases K^+ conductance either through increasing BK channel activity [122] or by opening a separate TREK channel [123,124]. In view of the complex effects of mechanical stretch on channel gating the overall significance of these responses remains unclear.

3. DETRUSOR ACTIVATION AND RELAXATION

In the normal human bladder acetylcholine is the sole neurotransmitter eliciting contraction, i.e. there are no atropine resistant contractions, whilst in many pathologies associated with bladder overactivity ATP is an additional activator [125-128]. Moreover age is also associated with atropine-resistant contractions [128]. With animal bladders, except for old-world monkeys, a dual muscarinic-purinergic activation is present. When muscarinic and purinergic receptors are inactivated there is generally no recorded neurotoxin (TTX)-dependent contraction, indicating that acetylcholine and ATP are the two major neuroactivators.

Despite the fact that M_2 receptors predominate over the M_3 subtype, most studies conclude that the latter mediates at least 95% of contractile activation [see 129,130]. More recently, the role of M_2 receptors has been re-evaluated. It has been advocated that M_2 receptors exert a more significant role in certain pathological conditions (eg, denervated or hypertrophied bladders), or when the M_3 receptor is desensitized [131-133], although this conclusion is not reached by all [134]. One possibility for this discrepancy is that M_2-dependent actions may derive from the urothelium [136], and that this pathway becomes more significant in these pathological conditions. The question arises as to what are the functions of M_2-receptors in the normal bladder and several studies indicate that they facilitate the function of other receptors, such as M_3 receptors [136], or counteract the relaxant effect of β-adrenoceptor agonists [137]. However, there is little difference on overactive bladder function between the effect of more selective M_1/M_3-selective blockers and those with a less specific action (balanced receptor blockers), although the side-effect profiles are different [138].

The major effect of ATP on detrusor smooth muscle is through ionotropic P2X receptors, generating depolarizing non-specific cation influx, which opens L-type Ca^{2+} channels to permit further Ca^{2+} influx [139]. The potency of the non-hydrolysable ATP analogue α,β-methylene ATP (ABMA) to elicit increases of intracellular [Ca^{2+}] was not different in cells from stable and overactive human bladders. $P2X_1$ labelling is present on detrusor [140] and it is presumed that ATP acts mainly through this subtype, although this exclusive route has been challenged [141]. It is of interest to note that $P2X_1$ receptor expression, in human bladder samples, is down-regulated with age [142], to offset the increased neurally-mediated release [128], although this is not a consistent observation [143]. More details of the purinergic activator system were detailed in the previous report [1].

Detrusor muscle relaxes in response to β-agonists (**figure 8**), mediated predominantly through a β_3-adrenoreceptor [144]. However, the physiological role of an adrenergic mechanism to control human bladder function is questionable. Despite this, the evaluation of β_3-selective agonists to reduce detrusor muscle tone represents an emerging subject [145-147], see also section VII.2.

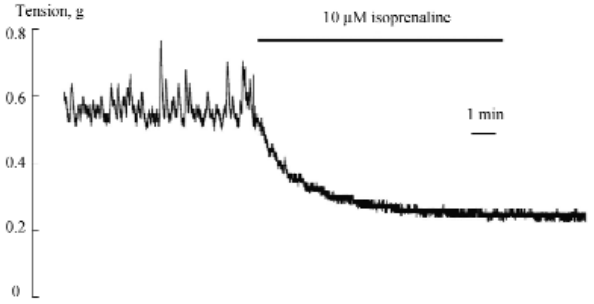

Figure 8: Relaxation of spontaneous and resting isometric tension in a rat bladder strip by the ß-agonist, isoprenaline. AJ Kanai unpublished data.

4. SPONTANEOUS ACTIVITY

Non-neuronal tetrodotoxin (TTX)-resistant contractions that occur in the detrusor have several aliases including: autonomous; intrinsic; micromotion; microtransient; non-micturition; phasic; rhythmic; spontaneous or transient activity. This activity was first reported by Sherrington in cats, as transient rises in bladder pressure seen during the filling phase [148]. Spontaneous smooth muscle contractions can also stimulate afferent fibres and generate centrally-mediated 'reflex' bladder contractions. These contractions, which are also referred to as non-micturition or phasic contractions, can be abolished with intravesical capsaicin without affecting smooth muscle-mediated spontaneous activity [149].

In neonatal rats, spontaneous activity, resistant to TTX, is absent at birth, increases in amplitude by week two, then changes from high-amplitude low-frequency to adult-like low-amplitude high-frequency activity by week six [70,150,151], **figure 9**. Micturition in neonatal rats is mediated by a somato-bladder spinal reflex pathway that is activated by the mother licking the perineum of the pup. During postnatal development, this primitive reflex is replaced by supraspinal mechanisms that control mature brain-to-bladder reflexes and voluntary voiding [152]. This developmental change in the central control of voiding occurs in concert with changes in peripheral neurotransmission and the spontaneous properties of the bladder smooth muscle [153-155]. Neurally-evoked bladder contractions are mediated entirely by cholinergic mechanisms in bladders from one-week-old rats, but become primarily purinergic in bladders from two-week-old animals. In bladders from spinal cord transected (SCT) rats [41] and outlet-obstructed

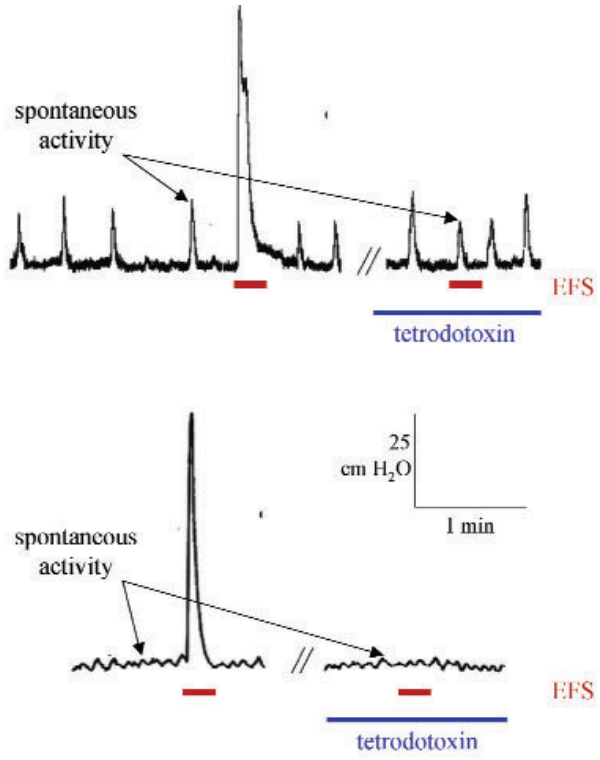

Figure 9 : Spontaneous and evoked activity in isolated rat bladders. A: a neonatal rat bladder showing large spontaneous contractions (arrowed) and larger contractions evoked by electrical field stimulation (EFS, 10 Hz) during the red bars. The EFS contraction, but not the spontaneous activity, was abolished by the neurotoxin, tetrodotoxin (TTX, 1 μM). B: a similar experiment in an adult rat bladder. The spontaneous activity is now of small amplitude, randomly fluctuating events. Modified from [70].

[142,143] bladders, there is a reemergence of high-amplitude low-frequency spontaneous activity similar to that observed in neonatal bladders.

1. The neurogenic hypothesis: reduced peripheral or central inhibition increases activation of the micturition reflex and contractions associated with detrusor overactivity [156].
2. The myogenic hypothesis: changes to the excitability and coupling of smooth muscle cells with other myocytes or interstitial cells leads to the generation of uninhibited contractions [157].
3. The urotheliogenic hypothesis: changes in the sensitivity and coupling of the suburothelial myofibroblast network leads to an enhancement of spontaneous detrusor activity [158].
4. The autonomous hypothesis: structures within the bladder wall coordinate to drive spontaneous contractions, which become enhanced in pathology [159].
5. A small leak of transmitter from motor fibres sufficient to cause small local contractions or increase tone [160].

The neurogenic hypothesis can account for neurogenic detrusor overactivity, but does not fully account for so-called sensory overactivity. However, it may well form a distinct subset of patients with detrusor overactivity because muscle samples taken from such bladder are no different from stable bladders and different from other non-neurogenic (idiopathic) overactive bladder samples [e.g. 127].

Spontaneous activity can be recorded from isolated muscle strips and several reports record an increase in samples from overactive bladders. Contractions are resistant to neurotoxins but generally labile to Ca^{2+}-channel blockers or K^+ channel openers [77]. Such activity can be recorded in isolated cells, as spontaneous changes to membrane potential and intracellular Ca^{2+}. Moreover the incidence of such activity is enhanced in cells isolated from overactive bladders. The origin of such activity is not known at present. Whilst up-regulated activity in isolated cells may be present, this will not alone explain spontaneous activity in multicellular preparations. Two possibilities have been proposed for increased intercellular communication. One is that there is an increase of intercellular coupling through gap junctions. Gap junctions are composed of the connexin (Cx) family of proteins. In human detrusor, expression of the main intermuscular connexin, Cx45, is actually less in samples from idiopathically overactive bladders and this correlated with a higher gap junction resistance in such samples [161]. Other groups however, suggest that another connexin isoform, Cx43, forms gap junctions between muscle cells and expression is upregulated in overactive bladders [162,163]. However, Cx43 labels interstitial cells in the detrusor layer although their number has not been reported to increase in overactive bladder models [77]. These cells are characterized by their labelling for the tyrosine-kinase receptor protein c-*kit* [160] – as are the suburothelial equivalents – close apposition to muscle cells and nerves [53], and the generation of spontaneous and carbachol evoked Ca^{2+} and electrical activity [164-166]. It is postulated that rather initiate spontaneous activity in the detrusor syncitium, interstitial cells modulate its activity [167], possibly by co-ordinating activity in different muscle bundles. However, these cells could form a control point for regulation of spontaneous activity, as they are innervated by nerves that label for nitric oxide synthase [55], and also express cGMP activity [168].

The urotheliogenic hypothesis is premised on a urothelial-myofibroblast network connected by gap junctions supporting pacemaker-driven spontaneous activity where bladder pathology, due to spinal cord transection for example, leads to an upregulation of gap junctions in the urothelium and myofibroblast network. This, in turn, leads to the formation of an increasingly functional myofibroblast syncytium with focal pacemaker activity that drives spontaneous contractions. The mechanism whereby this may drive

activity in the detrusor layer has been considered above (section II.3). **Figure 10** shows an experiment consistent with this hypothesis. Isometric tension recorded from detrusor strips, with the urothelium removed (top) or left intact (bottom). Both respond similarly to a maximum concentration of the cholinergic agonist carbachol, but in the bottom trace not only is there significant spontaneous activity but it is up-regulated by the purine UTP, that excites suburothelial myofibroblasts, but not directly detrusor smooth muscle.

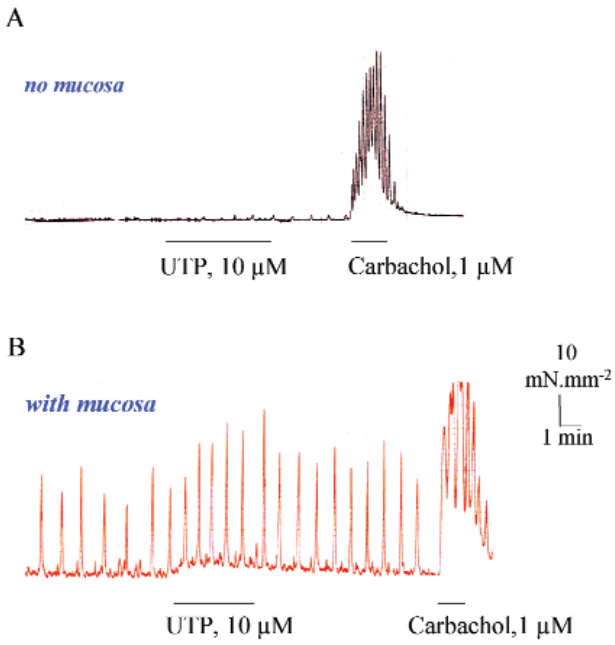

Figure 10: The influence of an intact mucosa on spontaneous activity in isolated bladder wall strips from guinea-pig bladders. A: a strip with mucosa removed; UTP and carbachol were applied as indicated by the horizontal lines. B: a similar experiment with a strip with an intact mucosa. Modified from [71]

The autonomous hypothesis takes into account the potential role of interstitial cells within the bladder wall but does not clearly indicate a specific mechanism by which spontaneous activity is driven. It most likely represents a qualitative description of a combined myogenic/urotheliogenic hypothesis. As with the urotheliogenic hypothesis spontaneous bladder contractions are enhanced by low-dose muscarinic stimulation, via M_3-receptors on suburothelial myofibroblasts. Activity is modulated by ATP, substance P, nicotinic receptor agonists, noradrenaline and nitric oxide, by unspecified mechanisms [169-171].

5. TRIGONE

The bladder base consists of the trigone, the urethrovesical junction, deep detrusor, and the anterior bladder wall. The outstanding developmental and functional position of the bladder trigone has been confirmed in decades of urological research and is defined as the triangular region situated between the ureteral orifices and the bladder outlet. It has always been considered to play a crucial role in ureterovesical function, continence and micturition.

Histologically, the trigone is characterised by smaller myocytes in smaller muscle bundles than in detrusor which exhibit extensive electrical coupling via gap junctions [172,173]; it also contains a greater amount of connective tissue than detrusor.

The original, and still prevailing, concept of a functional entity of bladder base, trigone, and ureterovesical junction, was first been developed by Waldeyer [174] in the late 19th century and later refined by Tanagho [175-177]. According to this concept, the spirally-oriented ureteral muscle fibres become longitudinal, as the ureter pierces the bladder wall obliquely, travels 15-20 mm, and terminates at the ureteral orifice. Fibres from each ureter fan out over the base of the bladder to form a superficial triangular sheet of muscle that extends from the two ureteral orifices to the internal urethral meatus and, from there, further down the urethra to insert at the verumontanum. The edges of this muscular sheet, the so-called superficial trigone, are thickened between the ureteral orifices (the interureteric crest or Mercier's bar) and between the ureters and the internal urethral meatus (Bell's muscle). A few centimetres from the bladder, the fibromuscular sheath of Waldeyer departs from the outer muscular layers, surrounds the prevesical ureter longitudinally, and continues in the bladder as the so-called deep trigone, which is fixed at the bladder neck. The trigone is backed by outer, longitudinal and middle, circular smooth muscle layers of the detrusor. In the space between Waldeyer's sheath and the ureter, only loose fibrous and muscular connections in the sense of a gliding plane are found. As the bladder fills, the bladder wall telescopes outward on the ureter, thereby increasing intravesical ureteral length. This facilitates passive occlusion of the ureter by the urine and warrants, like a flap valve, the basis for a reliable antireflux mechanism. This concept requires a competent vesico-ureteric anchoring, mainly provided by a strong contralateral ureteral smooth muscles blending in the shape of the interureteral ridge. Periodic contraction of this interureteric crest is thought to support the occlusive mechanism by further elongating the intravesical part of the distal ureter.

In fact, trigonal myocytes in the bladder base have recently been shown to exhibit marked spontaneous activity, which is mainly carried by Ca^{2+}-influx via membrane L-type Ca^{2+}-channels. Similar to interstitial cells, Ca^{2+}-actvated Cl—channels rather than K^+-channels contribute to the generation of spontaneity. Extensive gap junction coupling ensures electrical propagation and provides sustained spontaneous contraction of the interureteric crest [173].

Accordingly, the trigone is thought to develop, with the ureter, from an outgrowth of the mesonephric duct, and the common mesodermal origin of the vesical neck musculature, the trigone, and the ureterovesical junction is emphasised [175]. However, recent studies challenge this concept of a common developmental origin and suggest rather that the trigone is formed predominantly from bladder muscle; and the contribution from ureteral longitudinal fibers at the lateral edges that is much more limited than previously thought [178,179].

Within the lower urinary tract, the bladder base and trigone represent an area of dual parasympathetic-muscarinic and sympathetic-adrenergic innervation. Whilst mRNA measurements, western blotting, radioligand binding assay and receptor autoradiography studies have detected only low densities of α_1-adrenoceptors in the detrusor of several species, including humans, a more consistent, and in some comparative studies, greater α_1-adrenoceptor expression was observed in the trigone and bladder neck region. The α_{1D}-adrenoceptor seems to be the most abundant subtype in humans [180]. Reports about the proportion of adrenergic, muscarinic and other transmitter systems in the trigone are variable. Speakman *et al.* [181] found the maximum contraction to carbachol was no more than 50% of that elicited by phenylephrine (PE) in human tissue, but detected comparable reduction (40%) of electrically-evoked contractions by either muscarinic or α-adrenergic antagonists. Templeman *et al.* [182] showed a maximal responsiveness to PE of only 68.7% compared to carbachol in longitudinal strips of the pig trigone, when the urothelium had been removed. Roosen *et al.* [183,184] found a predominant muscarinic innervation and agonist responsiveness of the superficial guinea-pig trigone, with a reduction of electrically-evoked contractions by prazosin of 41.0% compared to atropine and mean maximal contractions to PE of 67.6% relative to those of carbachol. However, the functional significance of this dual innervation is much less clear. Whilst it is widely agreed in animal experiments that adrenergic stimulation via sympathetic nerves is active during the storage phase and induces contraction of the bladder base and internal urethral smooth muscle sphincter, the role of the parasympathetic innervation is much more controversial. Some propose that cholinergic axons exert a relaxing effect on urethral and bladder neck smooth muscle via generation of nitric oxide during micturition [185]. However, strip preparations from the bladder base have been shown to contract not only to carbachol superfusion, but also to the muscarinic component of electrically-released endogenous neurotransmitters. Moreover, there is a significant adreno-muscarinic synergism in the guinea-pig bladder trigone – **figure 11** - where the adrenergic pathway primarily operates through Ca^{2+}-sensitisation of the

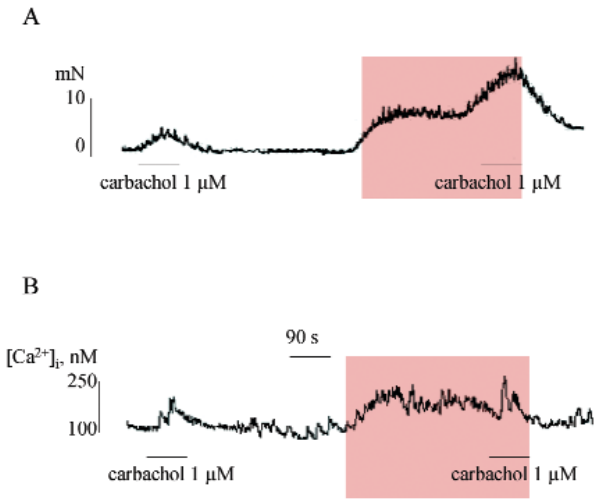

Figure 11: Effects of muscarinic and adrenergic agonists on the guinea-pig trigone. A: isometric tension from an isolated strip exposed to carbachol (1 µM). During the shaded region the strip was also exposed to phenylephrine (10 µM). B: a similar experiment from an isolated cell loaded with the Ca2+ indicator Fura-2. Modified from [180].

contractile machinery [183,184]. This is capable of a more than four-fold potentiation of muscarinic force activation which, in turn, seems to be a basically $[Ca^{2+}]_i$-dependent event. This synergistic mechanism might cause an even stronger contraction of the interureteric muscle in cases of involuntary detrusor contractions (induced by increased parasympathetic tone) and thereby prevent urinary reflux into the ureter.

If, as some suggest, the trigone takes part also in urinary outflow control, this synergy might lead to a higher closure pressure in cases of involuntary detrusor contractions and thereby prevent urinary leakage.

In general, the bladder base is thought to provide a rather stable structure against which the bladder dome can contract and relax during the micturition cycle. This might be the reason for the relatively high amount of connective tissue and the high spontaneous activity within individual myocytes. However, it is believed that during micturition the trigone may relax and cause a “funneling” effect to facilitate voiding. As in the urethra, animal experiments have shown that NO is the key relaxing factor in the bladder base [185-187].

NO effectively relaxes isolated smooth muscle preparations from the outflow region, suggesting that it may be involved in the decrease in intraurethral pressure observed at the start of normal micturition. This NO-based relaxation provides an effective mechanism to allow the bladder base and trigone to switch from a closed to open state, especially in combination with the adrenergic control of the Ca^{2+}-sensitisation of the contractile machinery.

IV. THE URETHRA

1. INTRODUCTION

During voiding, relaxation of the bladder outlet precedes detrusor contraction and during filling the outlet is contracted. Several mechanisms contribute to these functions, mediated through urethral smooth and skeletal muscle as well as the mechanical properties of the lamina propria. Inadequate closure of the urethra during filling could contribute to stress urinary incontinence. Smooth muscle is arranged as an outer circular layer and an inner longitudinal component, contraction of the circular should maintain continence, and longitudinal muscle may shorten during micturition. In women it has been suggested that there is greater dependence on surrounding support from the pelvic organs for the intra-abdominal portion of the urethra, resulting in the maintenance of an intra-abdominal portion of the urethra and buttressing against the fixed proximal portion of urethra, in combination with pressure generated by the urethral musculature. Therefore, the smooth muscle component may be more for maintaining a continence mechanism It has been estimated that as much as 50% of total urethral pressure in women is due to smooth muscle tone [188,189]. The urethra is innervated by both the sympathetic and para-sympathetic systems. Activity in pelvic nerve parasympathetic fibres relaxes urethral smooth muscle, especially in the proximal portion, and therefore the outflow region; sympathetic fibres (T10-L2) generate contraction.

2. URETHRAL SMOOTH MUSCLE

Sympathetic control is mediated by α_1 receptors, mainly the $\alpha_{1A/L}$ subtype in both human and animal models [190,191]. Partial $\alpha_{1A/L}$ agonists, such as Ro 115-1240, have been proposed as agents that may be used to manage stress urinary incontinence in women without significant cardiovascular side-effects [192,193], although their effectiveness remains to be demonstrated. α_2 receptor agonists induce contraction in several animal models but this has not been reproduced in human preparations [180]. Urethral relaxation can be mediated via β-receptor activation, predominantly the β-subtype. However, this mechanism is probably less important than in detrusor [180,194]. Of interest however is the observation that the $ß_2$-agonist clenbuterol also increased urethral skeletal muscle contraction raising the possibility that it may have a role in the treatment of urinary incontinence by inhibiting the detrusor contraction and augmenting external urethral sphincter activity [195,196]. Urethral relaxation during voiding is mediated by release of NO. Furthermore, prejunctional muscarinic receptors may limit noradrenaline release from sympathetic fibres, thus contributing to relaxation [197]. Muscarinic agonists generate urethral contraction, although receptor density is lower than in detrusor and clinical studies show little effect on urethral pressure [185].

NO-mediated relaxation is due to production of cGMP and activation of cGMP-dependent protein kinase, cGK. Electrical field stimulation generates relaxations that are abolished by inhibitors of NO production and are absent in mice lacking cGK [198]. The mechanism for relaxation does not seem to involve myocyte hyperpolarisation and awaits definitive evaluation [199]. CO can also exert relaxation through a rise of cGMP, which may be equivalent in magnitude to the effect of NO [200]. Derangements of the NO system have been demonstrated in several disorders associated with lower urinary tract function, such as diabetes, bladder outlet obstruction or bladder inflammation [201,202].

Sex hormones play an important role in modulating urinary tract smooth muscle function, including that from the urethra. Lack of oestrogen following menopause may contribute to decreased urethral tone, urothelial integrity and incontinence, and the older literature suggested that estrogen supple-mentation may be beneficial [203]. However, several recent clinical studies indicate that supplemental oestrogen lowers collagen content in the periurethral connective tissue and decreases urethral closure pressure [204-206] thereby worsening the symptoms of incontinence [207-209]. Similarly, recent studies in rabbits indicate that the response of urethral pressure to α-adrenergic agonists is equivalent between control, ovariectomized and ovariectomized rabbits with oestrogen replacement [210].

Urethral smooth muscle exhibits spontaneous electrical and mechanical activity that will contribute to the overall tone exhibited by the tissue. Electrical activity occurs as bursts of spikes superimposed on a slower more rhythmic activity, and could be initiated by autonomic transmitters [211]. Two types of Ca^{2+}-currents – L-type and T-type - have been recorded in isolated myocytes. Blockade of the former type reduced the number of spikes in each burst; the frequency within bursts was attenuated by blockade of T-type current [212].

However, both channels represent targets that may modulate spontaneous activity. The muscle cells are closely associated with interstitial cells that may be responsible for such activity, or at least modify it [165,213]. Activity in these cells results from intracellular Ca^{2+} release, itself triggered by Ca^{2+} influx, and the generation of Ca^{2+}-activated depolarising current that generates the electrical activity. The observation that interstitial cells are closely associated with NOS-synthase containing nerves suggests that they may be intermediaries between nerves and urethral smooth muscle [214].

3. URETHRAL SKELETAL MUSCLE

The skeletal muscle of the urethra wall (rhabdosphincter) forms an incomplete ring of skeletal muscle around the urethra [215]. In human tissue three fibre types have been described; fast-twitch fatigue-sensitive, fast-twitch fatigue-resistant and slow-twitch, with the majority fatigue-resistant [216, 217]. Muscle bulk decreases with age and also with parity in women [218, 219], **figure 12**. Stress urinary incontinence (SUI) may be associated with a loss of muscle mass in the urethra [218, 220], due to increased apoptosis and/or denervation [221]. Alternatively, a decrease in motor nerve function may also contribute to SUI as shown in a mouse model where vaginal distension with a balloon reduced leak point pressures and the number of nerves in the urethra, without affecting muscle mass [222].

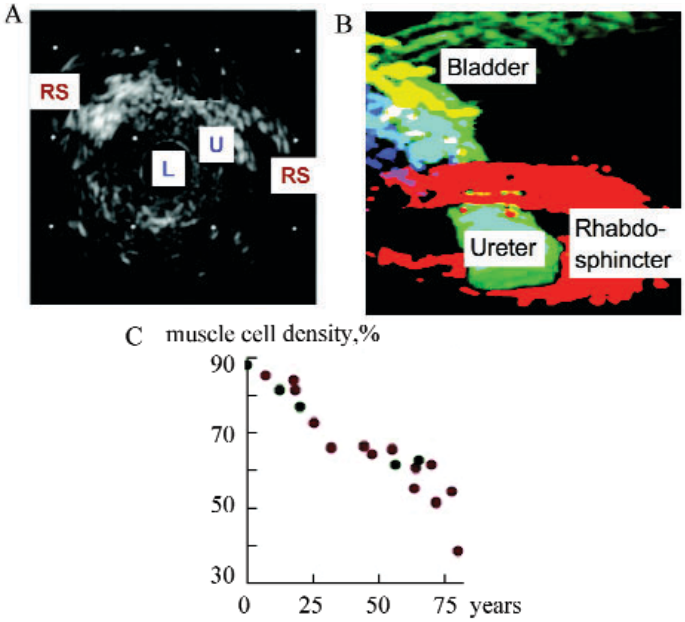

Figure 12: The skeletal muscle component (rhabdosphincter) of the urethra. A: transurethral ultrasound of the rhabdosphincter (RS) region of the urethra; the urethral mucosa (U) and lumen (L) are also labelled. B: a computer-constructed image of the rhabdosphincter component of the outflow tract in the male. C: the proportion of muscle cells in rhabdosphincter specimens from patients of different ages (brown, male; green, female).

The decline of sphincter, and striated muscle, function has been the motivation for the development of myoblast implants that may improve continence [223], or the use of basic fibroblast growth factor to facilitate muscle cell generation [224]. Subsequent work showed that when using implants, muscle fibre cells showed improved results over the use of fibroblasts [225]. It should be cautioned however that conclusions may depend on the variables measured to assess the effectiveness of any procedure. One study showed that urethral pressure was unaltered during filling cystometry, despite an increase of electromyographic activity from the urethral sphincter [226].

Selective inhibitors of serotonin (5-HT) and noradrenaline (NA) uptake, such as duloxetine, have been developed as potential agents for the therapeutic management of SUI [227], because 5-HT and NA terminals are present in Onuf's nucleus that supplies the rhabdosphincter with motor nerves [228]. Analyses of current data suggest that the effect of duloxetine on alleviating USI is small, but significant [229], thus accounting for its limited use.

The striated muscle of the urethral sphincter may undergo abnormal activity resulting in urinary retention, Fowler's syndrome [230,231]. The origin of the condition is unknown but one hypothesis is that it is due to ephaptic (i.e. direct cross-talk) electrical transmission between cells, much as can occur between nerve axons under certain conditions [232]. Neuromodulation may be effective in restoring voiding activity but there remain significant complication rates [233,234].

V. CELL PHYSIOLOGY OF NEUROACTIVE AGENTS IN THE LOWER URINARY TRACT

1. MUSCARINIC CHOLINERGIC TRANSMISSION

Acetylcholine is released not just from parasympathetic and somatic motor nerves to smooth and skeletal muscle targets respectively, but also from non-neuronal sources such as the urothelium. Muscarinic receptors are one of the main targets and are expressed throughout the lower urinary tract, nicotinic receptors are considered below. The muscarinic receptor family is divided into five subtypes based on molecular (m_{1-5}) and pharmacological (M_{1-5}) characteristics. Currently, the M_{1-4} receptors have been pharmacologically characterized, while an M_5-specific compound has yet to be developed [235]. In detrusor, immunoprecipitation analyses show m_2 and m_3 subtypes are expressed, with m_2 receptors in three- to nine-fold excess [236]. In normal human detrusor the minor M_3 fraction is responsible for contractile activation, although the M_2 component may modulate the overall response and become prominent in pathological bladders (see section III 3). M_3 receptors are coupled to $G_{q/11}$-proteins, which importantly activate the enzyme phospholipase-C (PLC) to convert membrane phosphoinositides to the second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 in turn releases Ca^{2+} from intracellular stores, after binding to an IP_3-receptor, to activate the contractile proteins. There is a body of experimental evidence to support the relevance of this pathway in detrusor: muscarinic agonists generate a rise of [Ca^{2+}] independent of membrane potential and release is blocked by the IP_3-receptor blockers TMB-8 [111]; and carbachol-potency is reduced by other IP_3-receptor blockers such as heparin and PLC

inhibitors [237,238]. Moreover inositol phosphate production mirrors tension generation in detrusor strips exposed to muscarinic agonists [239]. However other work casts doubt on the exclusiveness of this pathway, in part due to the relative ineffectiveness of other PLC inhibitors reducing carbachol-induced tension. It has been suggested that activation of the rho-kinase pathway by G-protein activation and of protein kinase C by DAG reduces the activity of myosin light chain phosphatase as so increases the Ca^{2+} sensitivity of the contractile proteins (see section III 1); the rise of intracellular Ca^{2+} was explained by activation of non-specific cation channels coupled to L-type Ca^{2+} channel activation [90,240-242]. Several attempts to reconcile the controversy have been attempted. Frazier et al [243] reasoned that different experimental protocols and PLC inhibitors used by different groups might partly be responsible and concluded that PLC activation was not important, whilst other have found considerable species differences in the relative importance of inositol phosphate and other pathways [244]. In addition, it must be cautioned that some of the data for these experiments rely on the use of compounds such ryanodine and cyclopiazonic acid (both Ca^{2+}-store inhibitors) that have poor penetration into muscle preparations, so that a lack of effect cannot always be taken as a lack of importance for a particular pathway. M_2 receptors are coupled to G_i-protein that reduces cAMP production by its influence on adenylate cyclase activity. It has been proposed that M_2 receptor activation inhibits the effect of other agonists that increase cAMP production, such as β-receptor stimulation. Recent reviews summarise the role of muscarinic-dependent pathways in the bladder and their relevance to contractile activation [130,245], and are summarized in **figures 13 and 14**. Muscarinic receptors are also localized on urothelial cells and myofibroblasts in the suburothelial and muscle layers (Section II.3) but where there role is less certain.

Presynaptic muscarinic receptors have also been described where it is proposed that they modulate transmitter release in either a negative ($M_{2/4}$) or positive (M_1 [246]) feedback mode. Knockout experiments indicate that M4, rather than M_2 receptors inhibit transmitter release [247].

2. ADRENERGIC TRANSMISSION

Adrenergic receptors are predominantly found in the bladder neck and trigone regions and are activated by release of noradrenaline from sympathetic innervations to induce contraction of the smooth muscle in these regions. In the detrusor adrenergic receptors are also present that induce relaxation. There are five distinct adrenergic receptor types; α_1, α_2, β_1, β_2 and β_3, with each being further divided into subtypes. The α_1 subtype predominantly induces the closure of the bladder outlet through contraction

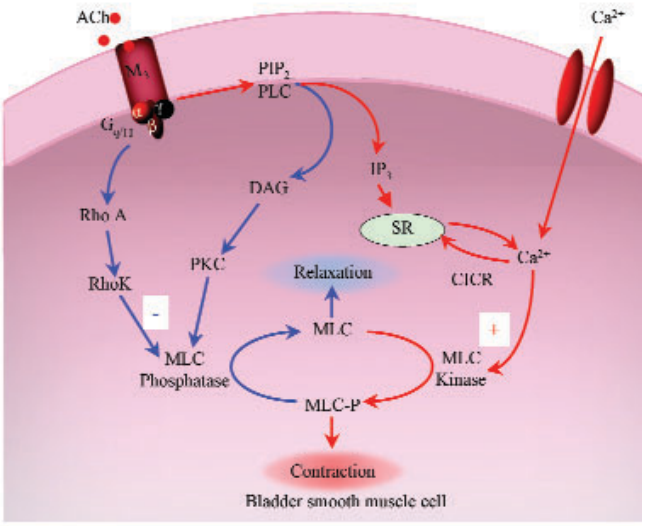

Figure 13 : Muscarinic, M3, signalling pathways. The major pathways initiated by acetylcholine binding to an M3 receptor are shown: i) the phospholipase-C (PLC) - inositol trisphosphate (IP-3); ii) diacylglycerol (DAG) – protein kinase-C (PKC) and iii) rho-kinase (RhoK) pathways. The influence of these pathways on the myosin light chain (MLC) kinase and phosphatase that determine contractile protein Ca2+ sensitivity are shown. Shown also is the route whereby transmembrane Ca^{2+} influx also determines the sarcoplasmic pool. CICR = Ca2+-induced Ca^{2+} release; SR = sarcoplasmic reticulum.

of the urethral smooth muscle, while prejunctional α_2-receptors modulate the release of neurotransmitters from sympathetic nerves There are also α_1-receptors (α_{1A} and α_{1D} subtypes) expressed on the detrusor smooth muscle, but they do not appear to mediate contractile activity [185]. However, noradrenaline can affect autonomous contractions of an isolated whole bladder preparation [171]. There is also evidence that α_{1D} receptors are present on the urothelium and may play a role in modulating reflex voiding [248].

All three β-subtypes are expressed in the detrusor smooth muscle with the β3-receptor being most highly expressed [249]. The β2 and β3-receptors can cause significant relaxation of trigonal and detrusor smooth muscle with apparent differences between species [250]. Furthermore, the effect is slightly smaller in hypertensive compared to normotensive rats [251]. There is much interest in specifically targeting β3-receptors for treatment of detrusor overactivity as it has a significant effect on reducing spontaneous detrusor contractions [252]. The β-receptor mediated relaxatory mechanism is thought to involve the rise of cAMP and modulation of large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channels [253] – see figure 14. β-receptors are also found on the urothelium where their stimulation can induce release of NO and may also be involved in the release of the urothelial-derived inhibitory factor [67]. A comprehensive survey of the *in vitro* and *in vivo* actions of β-receptor modulators has been provided [180].

3. PURINERGIC TRANSMISSION

The role of ATP as an extracellular signaling molecule is now well accepted. In most mammalian species ATP is co-released with ACh from parasympathetic nerves and activates purinergic receptors to initiate detrusor contraction. This is in contrast to healthy human bladders where contraction is predominately mediated by ACh. However purinergic nerve mediated contraction is increased in a number of bladder pathologies including hypertrophy, idiopathic overactivity, interstitial cystitis, neurogenic damage and aging (Section III.3). The increase of atropine resistance in bladder disorders may be due to; increased sensitivity of detrusor cells to ATP, increased release from motor nerves, or reduced ATP hydrolysis within the neuromuscular junction. The three possibilities have been investigated using human detrusor samples from stable and overactive bladders. Only the last hypothesis was considered a possibility as ectonucleotidase activity in overactive human bladder samples was significantly reduced [254]. The purinergic P2 receptors are divided into P2X and P2Y families based on pharmacological and molecular studies [255,256]. P2X receptors are ionotropic ligand-gated non-specific cation channels while P2Y receptors are metabotropic G-protein coupled. Currently seven P2X receptors subtypes have been cloned and characterized ($P2X_{1-7}$). $P2X_1$ receptors are the predominant subtype throughout the detrusor smooth muscle (**figure 14)** and activation generates an inward, depolarizing current sufficient to activate L-type Ca_{2+} channels to generate an action potential and Ca^{2+} influx to initiate contraction [139].

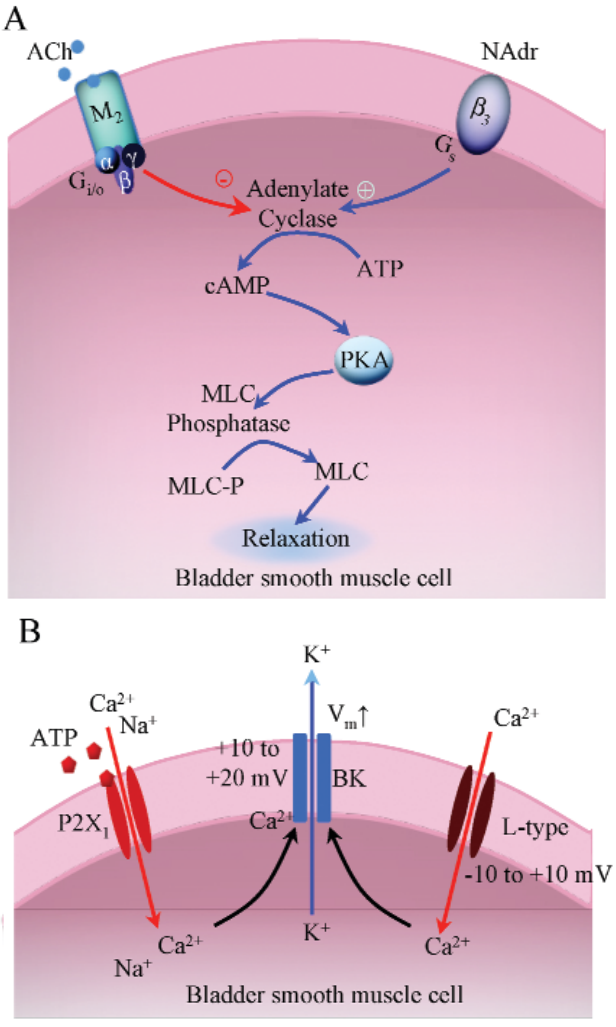

Figure 14: A; Muscarinic, M_2 and adrenergic ß3 signalling pathways. These pathways influence the intracellular cAMP levels, either increasing ($ß_3$) or decreasing (M_2) levels, through their influence on adenylate cyclase activity. cAMP, through a protein kinase-A (PKA) pathway, will promote relaxation by attenuating phosphorylation of the myosin light chain. B: Major ion channels in detrusor smooth muscle and their interaction. Two channels, the ionotropic P2X1 receptor and the L-type Ca^{2+} channel will conduct cation inward current thus depolarising the cell. The Ca^{2+} activated K^+ channel (BKCa) will conduct outward current.

All P2X receptors are present in cat bladder urothelium in the basal and apical layers [22]. Although the precise functional role of P2X receptors within the urothelium is still to be established, $P2X_2$ and $P2X_3$ may be involved in nociceptive signaling [257] and their expression has been shown to be up-regulated in painful bladder disorders including interstitial cystitis [258].

ATP released from the urothelium in response to hydrostatic pressure changes is believed to be important in bladder sensation and the initiation of micturition (section II.3).

There are eight subtypes of P2Y receptors ($P2Y_{1,2,4,6,11-14}$) linked either to $G_{q/11}$ ($P2Y_{1,2,6}$), G_i ($P2Y_{12-14}$) or several proteins: $G_{q/11}/G_i$ ($P2Y_4$); $G_{q/11}/G_s$ ($P2Y_{11}$) [259,260]. P2Y receptors, although not a specific subtype, have been implicated in relaxation of smooth muscle, possibly via cAMP-dependent PKA activity [86]. $P2Y_{1,2,4}$ have been identified on the urothelium [261]. Activation of P2Y receptors on urothelial cells from the rat evokes ATP release which may play a role in autocrine or paracrine signaling to modulate micturition [262].

$P2Y_6$ receptors have also been located on sub-urothelial myofibroblasts and respond to UTP with inward currents and large, transient increases in intracellular calcium [36,54]. In the cat $P2Y_4$ receptors were detected in nerve bundles close to the urothelium and detrusor smooth muscle, however their role remains to be established although activation of P2Y receptors could alter the release of neuropeptides through increases in intracellular Ca^{2+} [256].

4. NITRERGIC MECHANISMS

There are three nitric oxide synthase (NOS) isoforms, encoded by separate genes, named for the tissue that they were first isolated from or the order in which the genes were cloned: neuronal NOS (nNOS) or NOS 1; inducible NOS from macrophage (iNOS) or NOS 2; and endothelial NOS (eNOS) or NOS 3. There is also a form of nNOS that has a unique leader sequence that localizes the enzyme within mitochondria

(mtNOS). Each of these enzymes can be found in every cell type of the LUT. The expression of several factors determine if there is a relaxatory effect to nitric oxide: NOS; the NO receptor, soluble guanylate cyclase (sGC); and phosphodiesterase (PDE), the enzyme that degrades cGMP, the product of sGC activity. There are eleven PDE isoforms so far identified, PDE_{1-5} are described in the bladder [263-265]. PDE_5-selective inhibitors such as sildenafil (Viagra) and vardenafil are structural analogs of cGMP and competitively inhibit PDE. NO-donors have a rather small relaxatory effect on detrusor [266], but PDE inhibitors, such as vardenafil, relaxed pre-contracted detrusor [265], suggesting a relatively high endogenous PDE activity. These findings are corroborated by the beneficial effects of PDE_5 inhibitors with LUTS, when used to treat erectile dysfunction [267]. The importance of NO-mediated mechanisms in urethral relaxation has already been highlighted (section IV.2) [268]. The cellular pathways in contraction that are mediated by NO as shown in **figure 15**.

Capsaicin releases nitric oxide from the urothelium [28] and therefore may have effects on nearby cells in the suburothelial region including afferent nerves and myofibroblasts [168]. Nitric oxide has also been shown to free up tight junctions and disrupt the urothelial umbrella cell permeability barrier [269]. Accordingly, upregulation of iNOS during inflammation could compromise barrier function and exacerbate the pathology.

5. NICOTINE AND NICOTINIC RECEPTORS

Transmission at parasympathetic pelvic ganglia is mediated largely by nicotinic receptors [270], but there is also evidence for the involvement of nicotinic receptors at other sites in the lower urinary tract. Nicotine may evoke release of acetylcholine from motor nerves, which itself may be upregulated by tachykinins acting via NK2 receptors [271,272]. This contractile effect of nicotine is blocked by the nicotine receptor antagonist hexamethonium. The nicotinic receptor is a pentameter of subunits and to date 17 different subunits have been identified (α_{1-10}, β_{1-4}, γ, δ, ε) so that there is a large variety of potential receptors. Mutation studies suggest that the α_3 and β_4 subunits are required for bladder function [273], which is in keeping with the pentameter structure in autonomic ganglia – $(\alpha_3)_2(\beta_4)_3$.

Nicotinic receptor subtypes have also been localized to the urothelium, specifically for the α_3, α_5, α_7, β_3, and β_4 subunits [274]. Based on experiments infusing agonists and antagonists into the bladder lumen it was proposed that these subunits form two types of nicotinic receptor, one that increases and one that decreases bladder activity. Later studies have detected a wider range of subtypes with differential distribution to various layers of the urothelium [48]. Nicotinic pathways may also have a role in alleviating the effects of bladder inflammation. With artificial models of inflammation blockade of nicotinic receptors exacerbated the effect whilst activating the receptors produced the opposite effects, possible through an involvement of the cytokine IL-6 [275,276].

6. ADENOSINE RECEPTORS

Whilst purinergic, P2, receptors have been the subject of considerable scrutiny the pyrimidine P1 receptor family has received less attention. Four subtypes have been cloned, A_1, A_{2A}, A_{2B} and A_3 and all are G protein-coupled receptors, $A_{1/3}$ receptors are negatively coupled to adenylyl cyclase activity, whilst A_2 receptors are positively coupled. Adenosine relaxes bladder preparations pre-contracted by carbachol through an A_2 (possibly A_{2B}) receptor mechanism [277]. A_1 receptor binding was evident in a number of smooth muscle organs except bladder [278]. However, another study using the A_1 receptor agonist 2-chloroadenosine generated contractions linked to a PLC mechanism [279]. Using guinea-pig bladder preparations it has been proposed that A2-receptor

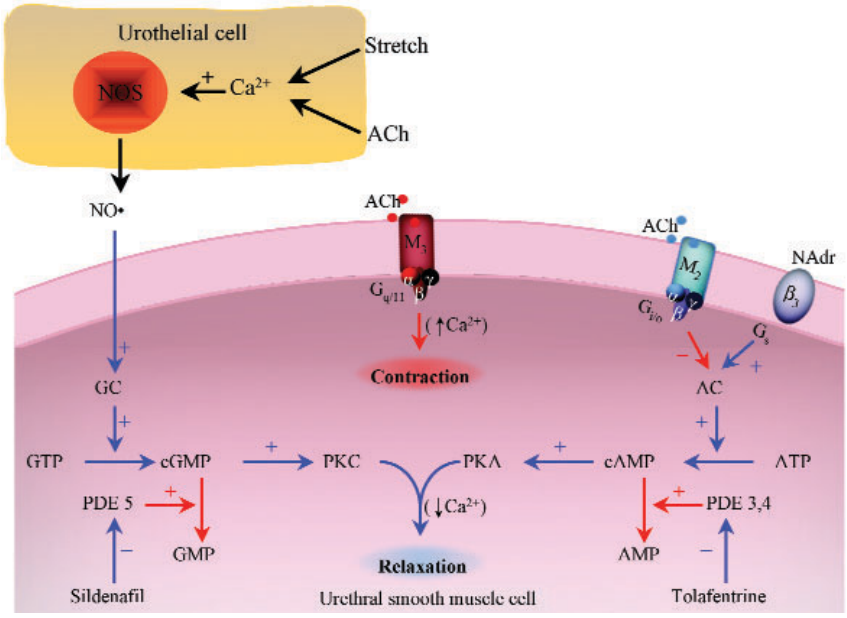

Figure 15: Nitric oxide (NO)-dependent pathways. Shown here is a scheme for a urethral smooth muscle cell where NO-dependent pathways are particularly important. NO is produced by activation of nitric oxide synthase and diffuses to a target cell, whereupon it increases cGMP production from GTP, by activation of guanylate cyclase (GC). cGMP, through a PKC pathway promotes relaxation. Muscarinic and adrenergic pathways are shown for completeness. Shown also are the sites of action of phosphodiesterase (PDE) inhibitors, sildenafil and tolafentrine.

relaxation is mediated by K_{ATP} channel activation, through increased adenylate cyclase activity, principally involving the A_{2A} receptor [280]. Adenosine also relaxes uretheral smooth muscle, but the receptor subtype is unclear [281]. The confusion regarding the involvement of particular receptor subtypes may in part be due to the evolving knowledge regarding the specificity of different receptor subtype modulators. All four subtypes are also localized to the urothelium with some differential distribution to the different layers. Adenosine is released from the urothelium, especially when mechanically stretched and it was hypothesized that adenosine receptors may mediate increase of umbrella cell surface area under bladder stretch [282].

7. NEUROPEPTIDES

Various neuropeptides, including calcitonin gene-related peptide (CGRP), substance P, neurokinin A, vasoactive intestinal polypeptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP), are released in the bladder from efferent and afferent [283] nerves and urothelial cells [284]. These peptides may also be released by noxious stimulus and promote inflammation. CGRP is an alternative product of the calcitonin gene expressed preferentially in nerve tissue. CGRP inhibits spontaneous activity and relaxes ACh-induced tension. Tachykinins (Substance P and neurokinin A) are prototypic of endogenous agonists of specific G-protein coupled receptors, termed tachykinin NK_1 and NK_2. NK_1 receptors have been found in blood vessels and the urothelium of all species thus far examined, whereas their expression in muscle cells seems restricted to rats and guinea-pigs [285]. The stimulation of NK_1 receptors activates phospholipase C leading to inositol phosphate accumulation and is linked to smooth muscle contraction. Substance P has stimulatory effects on detrusor smooth muscle and urothelium. NK_2 receptors are localized on detrusor muscle of all mammalian species studied, including humans [286,287], and in the suburothelial layer in rats. The stimulation of NK_2 receptors is coupled to inositol phosphate accumulation and is excitatory in the bladder. In the human bladder, tachykinin receptor type NK_2 predominates. VIP and PACAP receptors ($VPAC_{1/2}$ and PAC_1) are G-protein coupled receptors located on neurons and smooth muscle. They are coupled to several signal transduction pathways, including activation of adenylate cyclase and elevation of cyclic guanylate monophosphate levels in tissues [77, 288]. VIP release evokes relaxation of detrusor and urethra smooth muscle. There is indirect evidence to suggest that these various peptides can be released antidromically from afferent nerves in the bladder through different stimuli [289]. Potential stimuli for 'efferent-release from afferents' may be the intrinsic contractions associated with detrusor overactivity. However, whether this contributes to the enhanced spontaneous activity seen in pathology would depend on the peptide(s) released.

VI. BIOMECHANICAL PROPERTIES OF THE BLADDER WALL

1. ACTIVE AND PASSIVE CONTRACTILE PROPERTIES OF THE BLADDER WALL

The lower urinary tract is a hollow, neuromuscular system composed of four major layers: urothelium which lines the lumen; lamina propria; muscular detrusor and an outer serosal layer. The biomechanical properties of the bladder wall are principally dictated by the connective tissues of the lamina propria and the smooth muscle cells and connective tissues of the detrusor layer.

The detrusor, which comprises 60% to 70% of the thickness of the normal bladder wall, is composed of smooth muscle cells aligned in longitudinal and circumferential layers but are highly variable in cross-section, length and orientation; this orientation also changes during contraction [290]. Accordingly, the rise in luminal pressure during bladder contraction is not directly proportional to the sum of the muscle tension generated by cross-bridge formation in the myocytes. Some of the generated tension is absorbed by the connective tissues and only cells tangential to the radius of curvature of the wall contribute to force generation [291].

In principle, the different portions of the lower urinary tract need to exist between two physical states: high compliance, low wall stress, as exists in the bladder during filling; and high wall stress as exists during voiding in the bladder and in during storage in the urethra to maintain continence.

Overall wall tension results from the passive and active properties of the tissue; the former result from the viscoelastic properties of the collagen and elastin fibers in the extracellular matrices of the lamina propria and detrusor as well as the detrusor smooth muscle cells themselves. The active properties result from contraction of the component muscular structures within and surrounding the lower urinary tract and transmission of the resultant force through the extracellular and intracellular tissues. Whether the high compliance state is merely an absence of contraction of the muscular components or is contributed by active relaxation is a subject of increasing debate.

The translation of changes in wall tension to an increase or decrease of intraluminal pressure will also depend on the geometry of the system, i.e. the shape, intravesical volume and wall thickness. It is important to remember that whilst intraluminal pressure changes are the driving force for fluid movements in the lower urinary tract, the relationship with wall tension is highly dependent on geometrical factors.

2. COMPLIANT PROPERTIES OF THE BLADDER AND BLADDER WALL

There exists some inconsistency regarding the use of various terms to describe the relationship between distending or compressive forces and the resulting deformation. The stiffness, *k*, of a body is the ratio of an applied force to the resulting change of dimension; the inverse parameter is compliance and is a measure of the 'distensibility' of a system. In three dimensional terms pressures and volumes are substituted for force and distension, e.g. bladder compliance (*C*) is the ratio of change to bladder volume, *V*, per change to unit intravesical pressure, *P*, i.e. $C = \Delta V/\Delta P$. Generally, the elastic (Young's) modulus, *E*, is not the same as stiffness; *E* is a property of the constituent material, stiffness is an extensive property, i.e. a property dependent on the material and the geometrical shape of the body. Here compliance and stiffness will be used.

In principle, it is difficult to state a standard value for compliance for several reasons: bladder capacity increases with age whereas intravesical pressures do not vary as much; intravesical pressure itself is a function of bladder volume (see below) and thus compliance would vary as the bladder fills. Furthermore, it must be emphasised that compliance is a steady-state property so that measurements should only be made when any stress-relaxation has fully subsided. Several approaches have been attempted to account for these confounding factors. A normalization factor has been introduced in urodynamic studies to correct for values calculated in different size bladders [292,293]. Ex vivo, non-linear pressure-volume relationships have been transformed into linear stress-strain relationships to correct for different initial bladder volumes [294], when more direct comparisons can be made with data from tissue strips [294,295], **figure 16.**

Outflow tract obstruction in human and animal models has variable effects on bladder and isolated strip compliance. With shorter periods of obstruction compliance does not seem to alter [296], whilst after more extensive periods compliance may either decrease [297-300] or increase [294,295,301]. Most likely the different effects result from the severity and duration of the obstruction, with the bladder progressing from a hypertrophied low compliance state to a high compliance state that may mark end-stage failure [302]. The physiological consequences of this progression of function need to be considered. A decreased compliance will mean that intravesical pressures should rise more for a given amount of filling and if maintained can contribute to upper tract damage [303]. One consequnce of a greater compliance will be that detrusor contractions will be less effective in raising pressure and thus make voiding more difficult [289]. It is thus important to determine which physical properties of the bladder wall determine changes to the compliance of the bladder.

3. BIOMECHANICAL PROPERTIES OF LOWER URINARY TRACT TISSUES AND COLLAGEN SUBTYPES

The passive properties of LUT tissues will depend on the respective properties of the muscular and extracellular components and the respective proportions of each [291,304]. The most significant extracellular components in this context are collagen and elastin; of the former collagen types I and III have the most influence on mechanical properties [305].

In obstructed bladders collagen type-I and type-III content rises in the lamina propria, but particularly in the detrusor muscle layer, with an increased ratio of type-III to type-I reported in some [306-309]. Such increases of collagen may be accompanied by reduction of elastin gene expression [310]. Collagen

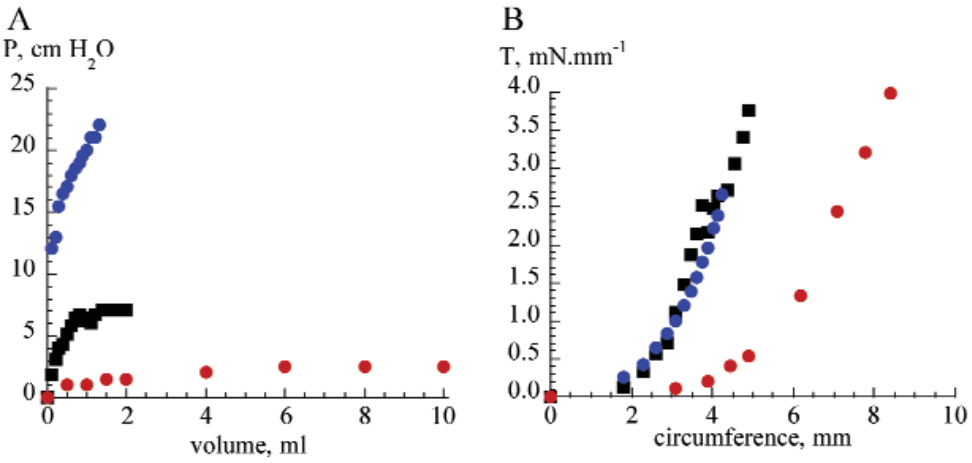

Figure 16: Bladder pressure-volume and derived tension-length relationships. A: ex vivo pressure-volume relationships for three different bladders from fetal sheep: red and blue symbols, obstructed bladders; black symbols, unobstructed control. B: derived tension-circumference plots from the data in part A. Tension was calculated according to Laplace's Law (see text for details) and circumference was calculated from bladder volume assuming a spherical shape. MK Farrugia, M Godley, P Cuckow and CH Fry, unpublished data.

I forms large fibers and predominates in tissues with high tensile strength, whilst collagen III, forming smaller fibers, imparts increased flexibility to tissues and an ability to rearrange during bladder filling [311]. This may be of particular importance to bladders undergoing hypertrophy with a large increase of muscle mass. Collagen synthesis by cells is upregulated by physical stretch, which may provide the basic stimulus in bladder outflow obstruction [312] and may be determined by an increased release of basic fibroblast growth factor from the urothelium [313]. During bladder development the collagen III:I ratio shows an inverse relation to compliance [314]; however other geometrical factors, as mentioned above, will also determine compliance so that changing collagen ratio *per se* is only one factor among many. The biomechanical properties of the bladder wall will depend not just on the quantity and type of collagen, but also on its packing arrangement, and much work is required to determine the inter-relationship of these factors in determining overall tissue compliance. **Figure 17** illustrates information that may be obtained by X-ray analysis on collagen packing arrangement.

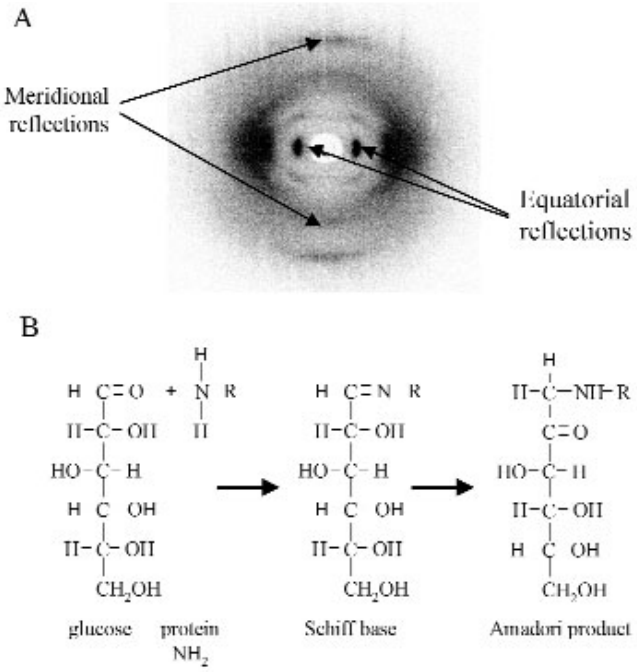

Figure 17: Collagen visualisation in whole tissue and formation of glycation end-products. A: wide angle X-ray diffraction pattern of hydrated rat tendon. Meridional reflections are from helical layers in the longitudinal axis of the collagen molecules; equatorial reflections from lateral packing of collagen molecules. B: the reactions of non-reducing sugars, such as glucose (shown here in open-chain configuration as in aqueous solution), with amine groups, such as lysine, on protein molecules (R). The initial steps involve production of a Schiff base (a functional group that contains a carbon-nitrogen double bond with the nitrogen atom connected to an aryl or alkyl group) and an Amadori rearrangement product. Such products can then combine causing cross-linking between protein molecules leading to disorganization of protein structure.

Environmental and other factors can have significant effects on the passive properties of the bladder. For example oestrogen deprivation reduced the smooth muscle, and increased the collagen content of the bladder wall [210]. The adduction (combination) of sugar to protein without the action of an enzyme (glycation or non-enzymic glycosylation) is associated with diabetes and ageing and a significant factor in damage to extracellular and cellular proteins by promoting cross-linking and aggregation. Figure 17 shows the first steps whereby a reducing sugar, such as glucose, reacts with a basic residue on a protein to form a Schiff base and Amadori product. The subsequent interactions of these products generates cross-linking between adjacent protein chains and a loss of integrity and function. This formation of advanced glycation end-products increases the stiffness of tissues [315]. However several studies have shown that bladder compliance actually increases in animal models of diabetes [316,317] so that the exact relation of this condition and the formation of advanced glycation products remains unclear.

Stress-relaxation is a feature of the whole bladder and isolated muscle strips. It is a visco-elastic phenomenon that manifests itself as a partial reduction of stress (pressure or tension) after a rapid change of strain (volume or length). Physiologically this is advantageous to the filling bladder as it enables steady-state changes of pressure to be minimized during filling. Practically it means that if a bladder is rapidly filled then changes to intravesical pressure may be greater than during slow-fill and give the impression that compliance is less than it actually is. Compliance is a steady-state measurement and changes to pressure or tension should only be made when any stress-relaxation has finished. Stress-relaxation may reside from a rearrangement of the cellular and extracellular elements in a muscle component, although isolated cells also show the same phenomenon indicating that internal rearrangement of cytoskeletal elements must also occur [318]. In the over-compliant obstructed bladder the extent of stress-relaxation diminishes in the same proportion as steady-state stiffness [295]. Because the proportion of extracellular material increases in these bladders this suggests that the phenomenon may reside in both the cellular and extracellular components.

Detrusor muscle also undergoes a phenomenon of strain-softening: this is a reduction of steady-state stiffness on stretch to a new length, distinct from viscoelastic behavior [319,320]. Different stress-strain relationships are illustrated in **figure 18**. When the muscle is relaxed the process is irreversible, possibly due to cross-link breakages and can take many minutes, even hours, to develop. However, when active force is generated the process is reversible as new cross-bridges are made. Thus the passive

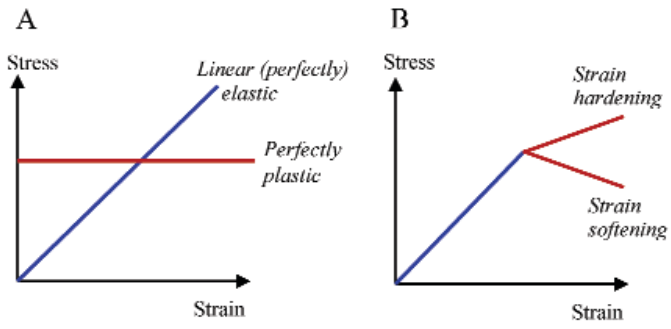

Figure 18: Stress-strain relationships. A: stress-strain relationships for a perfectly elastic and perfectly plastic system. The stiffness (inverse compliance) is defined at the ratio of stress/strain. B: Strain-softening and strain-hardening that is exhibited in some materials beyond the limit of perfectly elastic behaviour.

stiffness of detrusor depends on its previous history and is less if the tissue had been strain-softened – compliance would thus be less in a bladder that developed active force less frequently. Strain-softening is likely to be a cellular process as it is abolished by rho-kinase inhibition [321] – an intracellular pathway that increases the Ca^{2+}-sensitivty of the contractile apparatus.

4. PROPERTIES OF THE PELVIC FLOOR AND GENUINE STRESS INCONTINENCE

The support of muscle under the urethra provide a continence mechanism against which the urethra can be compressed during a rise of abdominal pressure, and the stiffness and mobility of this structure determines the extent to which compression can occur [219]. This is particularly important in women and it is in them that the majority of studies investigating tissue changes associated with sphincteric urinary incontinence have been carried out. In pre-menopausal women with stress urinary incontinence (SUI) the collagen content of the periurethral musculature is decreased [322-324]. One study showed however that the rate of collagen synthesis was similar in fibroblasts cultured from samples from women with or without SUI [325], so that reduced collagen content may be related to increased breakdown [326]. SUI is also associated remodeling of the tissue with respect to elastin-collagen interaction [327]. Elastase activity is also increased in pelvic floor tissue from women with SUI [328]. With post-menopausal SUI women the picture is less clear, with some studies showing a decrease [322,329], and others no change to collagen content [205]. However, greater cross-linking of fibres was observed in these SI women, which would decrease the flexibility of these tissues, except when they had been undergoing oestrogen therapy [330]. Some studies have indicated down-regulation of oestrogen receptors in tissue samples of women with SUI [331], but meta-analyses do not provide a strong case for oestrogen treatment [332]. Thus far, to our knowledge, no systematic studies of the biomechanical properties of pelvic floor tissues from women with and without SUI and their correlation with the composition of the extracellular matrix have been carried out.

5. THE ACTIVE PROPERTIES OF MUSCLE IN THE LOWER URINARY TRACT

The basic principles of muscle mechanics and a description of the contractile proteins in smooth muscle generally have been described in the previous report [1]. The role that intracellular Ca^{2+} plays in activating the contractile machinery was also considered previously and is up-dated in the section on the cell physiology of detrusor smooth muscle (III.3;V.I).

a) Length-tension relationships

All smooth muscles exhibit a bell-shaped dependence for active force on its initial resting length, ie there is an optimum resting length, L_0, at which active force is maximal. However, this length-tension curve extends over a greater range of initial lengths than does striated muscle. This is particularly true of detrusor where passive extension much beyond L_0 does not greatly reduce force [333]; human detrusor exhibits similar curves to those from animal preparations [334]. This has obvious physiological advantage whereby large changes in bladder circumference will still permit adequate force development by individual myocytes. Changes to tissue length are reflected in changes to cell length, so that these curves reflect accurately the length-dependence of contractions from individual myocytes [335,336]. There is evidence that length-tension relations are shifted rightwards, i.e. to longer lengths, in samples from some overactive bladders [337], but when normalized to L_0 curves do not vary much between control and pathological organs [338].

b)The contractile state

There is divergence of opinion whether absolute force (normalized to cross-section area) varies in samples from normal and pathological bladders. This is an important issue as it is important to know if conditions such as detrusor underactivity or overactivity are mirrored by changes to the contractile state of detrusor muscle. Alternatively they may be due to others causes such as alteration (total or proportional) to the amount of muscle in a bladder sample, the extent of functional innervation, or the physical properties of the extracellular matrix. Therefore, in this respect care must be exercised in the interpretation of experimental data, as contractile responses evoked agents such as muscarinic receptor agonists may give different responses compared to nerve-mediated responses. In the latter, a reduction of force may be due to denervation rather than muscle failure. To measure the relative extent of denervation compared to contractile

failure one method is to calculate the ratio of tension evoked by nerve-mediated stimulation and an agonist such as carbachol. A reduction of the ratio would imply denervation [339]. Animal models of bladder hypertrophy due to obstruction suggest some contractile failure [295,340,341], but not in all cases [294] and it may be hypothesised that failure is a feature of later growth when a decompensated, more compliant bladder is generated by obstruction. With human detrusor there is little evidence that agonist-induced force is different in detrusor from normal and overactive bladders [127]. There have not been systematic studies of the contractile state of detrusor samples from underactive human bladders. Perhaps the most interesting task in this context is to determine the basis of the condition described as detrusor hyperactivity with impaired contractile function – a symptomatic condition [342] that describes bladder overactivity with incomplete emptying. It is ascribed to a reduction of detrusor contractility [342,343] although this is a description based on urodynamic measurements rather than one derived from principles of muscle mechanics.

6. TENSION AND PRESSURE

Contraction of the muscle component of a hollow system such as the lower urinary tract generates a wall tension (stress) that manifests itself as a change of internal pressure, i.e. energy per unit volume, and by Pascal's principle is everywhere the same in a static system. The quantitative relationship between pressure, P, and wall tension, T, is not linear but governed by Laplace's Law, which for a uniform cylinder or radius, r, and wall thickness, d, is given by: $P=2Td/r$. Thus a large radius sphere generating the same unit wall tension as a smaller sphere will undergo a smaller pressure change. This can lead to confusion when changes of pressure, regardless of the size of the vessel, are equated linearly to changes of wall tension and muscle performance. Two situations exemplify the need to understand the inter-relationship between pressure and wall tension: i) changes to internal pressure by passively filling the bladder; ii) calculation of active wall tension from internal pressure changes. The first situation [294] shows that when pressure-volume relationships are generated during bladder filling the plots are non-linear and are crucially dependent on the volumes used to fill the bladder. When normalised to wall tension versus changes to bladder radius the plots are linearised, enabling the compliance or stiffness to be calculated (compliance here is change of radius required to generate a unit change of wall tension). The second approach, to estimate active contractile properties of the bladder from pressure changes has been attempted from urodynamic measurements [344] and has been used to compare contractile properties of the bladder in patients with different pathologies [345]. However, this approach is really only useful when the pressure changes manifest wall tension changes alone and not when the energy in the fluid volume is being used to move fluid – i.e. when the pressure is generated isovolumically [346]. Furthermore, changes to contractility can then only be evaluated when muscle length (approximated by bladder radius) is constant, otherwise changes to the length-tension relationship will have occurred to confound the active tension estimations. **Figure 19** shows calculations of pressure - left - for a bladder contracting isometrically (constant volume) at two different volumes during development of wall tension. The larger volume bladder develops pressure. Shown also is the change of pressure during the development of wall tension when a bladder is emptying, or remaining at constant volume. In the

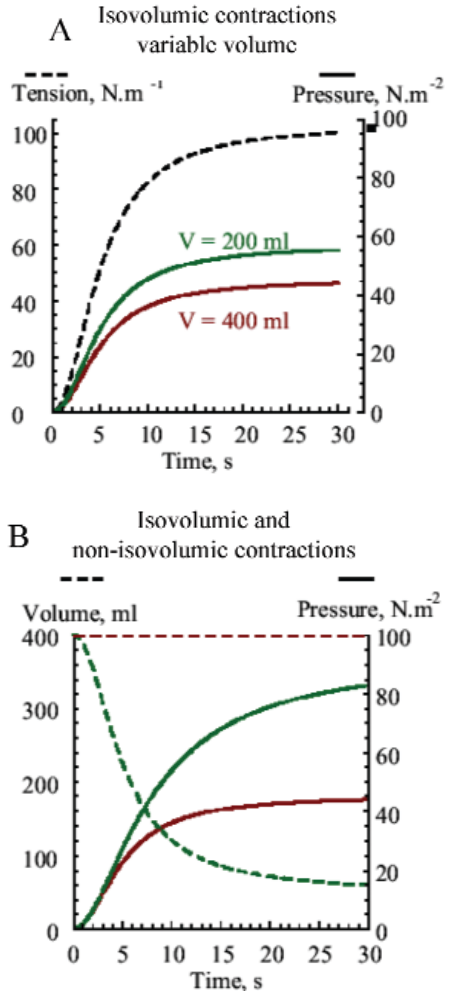

Figure 19: Contraction and pressure changes in a hollow organ. A: tension is developed in a unit element of the wall of a spherical organ as a function of time (dotted line). The solid lines show that consequent change of isovolumic pressure at two volumes, 200 and 400 ml. For the same amount of tension developed, the internal pressures are different. B: Pressures developed in a spherical organ due to the same change to tension as in part A. For the brown curves, volume (dotted line) remains contant; for the green curves volume reduces as the organ empties. Note again the different pressure profiles.

latter case more pressure develops as the bladder empties, because the radius is reducing as a function of time. The other extrapolation drawn from muscle mechanics is to estimate changes to the force-velocity relationship from pressure-flow studies. Again a superficial resemblance exists between the two relationships but often calculations fail to recognize the non-linear relationship between force and pressure, and also between velocity and flow, is the cross-sectional area through which the flow occurs is not constant. Attempts to review the various paradigms that relate urodynamic to muscle dynamical properties have been made [347-350], and further hydrodynamic analyses of the lower urinary tract are awaited.

VII. THE LOWER GASTRO-INTESTINAL TRACT

1. THE NORMAL PHYSIOLOGY OF THE RECTUM AND ANUS

The ano-rectum is a functional structure that maintains faecal continence and also facilitates defaecation, when appropriate. The structure is shown in **figure 20.** Continence is maintained by maintaining an adequate rectal capacity at pressures less than that required to overcome the resistance offered by a competent anal sphincter, analogous to the condition in the lower urinary tract. Anal sphincter competence is due to a combination of internal sphincter tonic contraction, augmented by voluntary control via the external sphincter [351]. The anal sphincter mechanism may be helped by a sling effect of the puborectalis muscle around the anal canal, and the magnitude of the anorectal angle correlates with the severity of incontinence [352]. Faecal incontinence is increased in patients where the muscle has been divided; however, results from procedures that generate an acute anorectal angle are inconclusive [353]. Rectal compliance is maintained on filling by a reflex decrease of tone. The ano-rectum contains the necessary systems of receptors, neural networks and pacemaker cells to maintain the filling and emptying functions, assisted with external autonomic and somatic control.

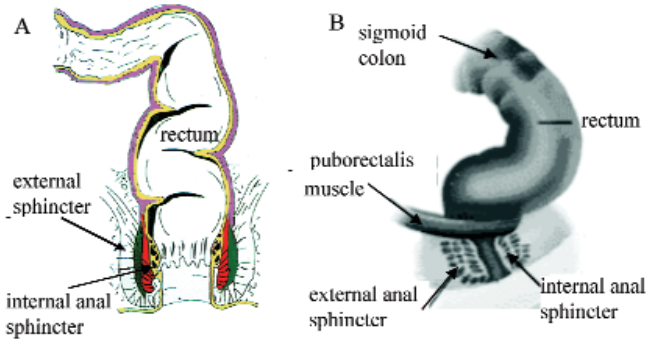

Figure 20: The structure of the ano-rectum. A: the internal and external sphincters are marked. B: the puborectalis muscle is illustrated.

The rectum is normally empty, but fills as faecal material accumulates in the descending and sigmoid colon, and is pushed forward through occasional peristaltic waves (mass movements). Volume of first sensation is up to 70-90 ml, urgency develops above about 200 ml [354,355]. A recto-anal sampling reflex [356] occurs on rectal distension that lowers pressures [357-359] in the anal canal allowing the contents to approach the mucosa, whilst the external sphincter remains contracted. The considerable sensory innervation of this region can distinguish between the gaseous (flatus) or solid state of the material. If defaecation is inappropriate contents are returned to the rectum, assisted by an increase of rectal compliance and inhibition of descending pathways. Such a sampling reflex can normally occur four to ten times per hour [360]. With larger volumes in the rectum, defaecation can be voluntarily deferred by contraction of the external sphincter and puborectalis (**Figure 21**).

If defaecation is appropriate intra-abdominal and intra-rectal pressures are raised to overcome sphincter resistance; the process is assisted by reflex relaxations of the internal and external sphincters, as well as the puborectalis. An increase of the anorectal angle also facilitates the process; brought about by taking up a sitting or squatting position and puborectalis relaxation. Upon completion, the internal sphincter and puborectalis transiently contract and restore the anorectal angle.

Continence is therefore maintained by a number of factors:

- effective anal sphincters and an acute anorectal angle to provide a proper barrier
- a suitably large, compliant and evacuable reservoir
- intact sensation in the ano-rectum
- proper consistency of the faeces

The most common cause of incontinence is damage to, or dysfunction of, the external and internal anal sphincters or their nerve supply. In addition, damage to sensory nerves that detect stools in the rectum will derange the reflexes that maintain continence. Damage may occur during childbirth, pelvic tumours, haemorrhoid surgery or neurodegenerative conditions. A decrease of rectal compliance can occur following radiation treatment or inflammatory bowel disease. Other manifestations of pelvic floor dysfunction such as rectal prolapse, rectocoele or weakness of pelvic floor muscles will all increase the likelihood of faecal incontinence.

2. INNERVATION

Parasympathetic innervation to the rectum and anal canal, via the pelvic plexus, originates mainly from sacral segments S1-S4 (pelvic splanchnic nerves)

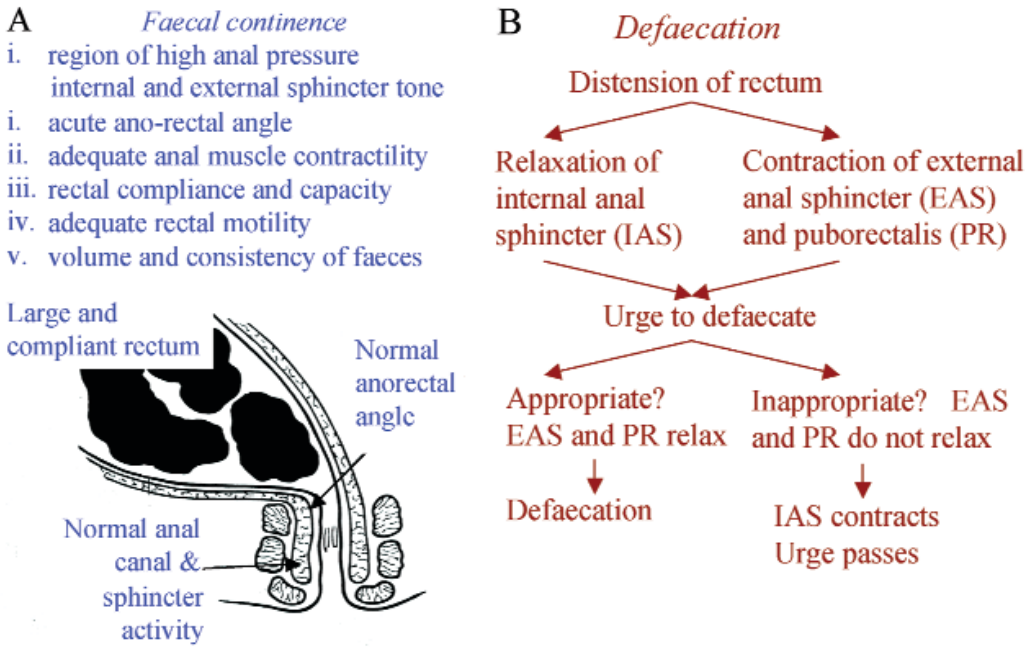

Figure 21: Components of faecal continence and defaecation. A: the conditions required for faecal continence. These include: appropriate muscle function in the anus and rectum to maintain a closed outlet and compliant reservoir; appropriate structural features; and proper consistency of the faeces. B: The stages in (deferred) defaecation.

[361]; the sympathetic supply is from T11,12 and L1,2. These fibres have the usual antagonistic effect on function with parasympathetic fibres increasing peristalsis and secretions. Somatic fibres to the external sphincter and pelvic floor muscles originate from Onuf's nucleus in S2-S4 and run in the pudendal nerve. Somatic and visceral afferents also arise from the ano-rectum: somatic fibres accompany the pudendal nerves and visceral afferents the parasympathetic and sympathetic efferents. The target for the postganglionic fibres can be the enteric nerve plexi that lie between the smooth muscle layers or the smooth muscle cells. Moreover there is increasing evidence that interstital cells of Cajal (ICC – see below) are the main target for excitatory and inhibitory motor neurones that modulate smooth muscle contractility.

The density of neurones in the myenteric and submucosal plexi declines towards the distal rectum and into the anus, but do not disappear together [362,363]. However, the variability of neuronal ganglia is variable along the ano-rectum and between patients and thus renders difficult the generation of criteria to neuronal deficits akin to intestinal neuronal dysplasia [364]. The use of botulinum toxin A is increasing advocated to manage various spastic conditions in the gastrointestinal tract [365,366], but as with its use in the lower urinary tract, its mode of action is unclear, although it has been postulated to diminish sympathetic activity [367]

Hirschsprung's disease is a developmental disorder of the enteric nervous system, characterised by an absence of ganglion cells resulting in functional obstruction. The agangliosis begins at the anus and continues proximally. The absence of ganglion cells results in an increase of extrinsic innervation, in particular adrenergic innervation, resulting in an increase of muscle tone and a functional obstruction. This pathology may be exacerbated by increased myogenic tone and loss of inhibitory control mediated through ICC [368,369]. The genetic basis of the disease has been investigated, and one hypothesis is that it is a failure of neural crest cells to migrate. A number of mutations have been identified including the proto-oncogene *RET*, an associated protein *EDNRB*, as well as various endothelin-receptor genes [370-372].

3. SMOOTH MUSCLE TISSUES

The rectum. The rectum is composed of outer, longitudinal and inner, circular layers of smooth muscle, as in proximal regions of the G-I tract, and generate phasic rhythms at 10-20 per hour [373]. The circular muscle develops little intrinsic tone, in contrast to that developed by the longitudinal muscle layer. Relaxation is induced by both alpha- and beta-receptor agonists with the latter generating more long-lasting responses [374]. Experiments with isolated strips show that nerve-mediated contractions are mediated by cholinergic (M_3) and non-cholinergic components, with little sympathetic, adrenergic activation [375]. The non-cholinergic fraction is importantly mediated by tachykinins acting on NK_2 receptors. The sympathetic supply to the rectum exerts an inhibitory influence, as cutting the thoraco-lumbar supply reduces motility of the rectum [376,377], which may by a presynaptic effect on excitatory nerves analogous to their action in lower urinary tract. In addition, the rectum receives a nitrergic innervation that relaxes the smooth muscle, as evidenced in preparations from human and animal sources [378,379]. 5-HT receptors

also mediate smooth muscle tone and peristalsis throughout the gastrointestinal tract. In human and canine rectum preparations relaxation is also mediated via $5HT_4$ receptors [380-382]. This is of interest because $5HT_4$ receptor-agonists might be used as modulators of rectal tone as they have uses in the management of gastro-intestinal disorders [383], although reported cardiac side-effects have resulted agents such as cisapride being withdrawn from the North Americal market [384]. More recent prokinetic receptor agonists (eg ATI-7505) may have fewer cardiac side-effects and so may prove safer alternatives [385].

The anal canal. The longitudinal smooth muscle layer of the rectum extends into the anal canal, to form a conjoint longitudinal coat, whilst the circular layer forms the internal anal sphincter. The responses to autonomic transmitters differ from the rectum whereby the anal sphincter muscle is relaxed by muscarinic and β-adrenoceptor agonists and contracts to α-adrenoceptor agonists. The longitudinal muscle contacts to both α- and β-adrenoceptor agonists [386]. The response to adrenoceptor agonists reflects the predominant effect of extrinsic symapathetic fibres in maintaining control of the anal sphincter [387]. Parasymapthetic fibres have been suggested to modulate sympathetic nervous activity [388]. Nitrergic fibres offer the predominant relaxatory tone and may exert their effect via the enteric nerve system (below) [389]. Oral intake of the NO precourser L-arginine had no effect on anal pressures [390], but topical application of L-arginine paste to anal fissure did ameliorate the condition [391]. The different responses along the anorectum may in part be due to the variation of receptor populations in this region [392]. Angiotensin-II (AT) has been proposed as an important mediator of tone in the anal sphincter and ACE-inhibitors as well as AT_1 receptor antagonists reduce internal anal sphincter pressures [393-395].

The distribution of receptor subtypes in the anorectum is of interest because of the desirability of developing more tissue selective agents to modulate muscle function. β_3-receptor subtypes have been demonstrated throughout the gastrointestinal tract in both human and animal preparations [396,397], and this may provide a role for receptor modulators as is being advocated in the lower urinary tract. Muscarinic responses are predominantly mediated by M_3-subtype receptors.

There has been considerable interest in the role of the rho-kinase system in maintaining and modulating tone in the anal sphincter [398], and has been proposed as a potential therapeutic target [399]. This pathway may be involved in the relaxatory effect of the PDE_5 inhibitor, sildenafil on precontracted anal smooth muscle [400,401]. Such pathways may be useful in the management of anal fissure, but an understanding of this mechanism will also be valuable in the investigation of the basis of faecal incontinence.

4. INTERSTITIAL CELLS

Interstitial cells of Cajal (ICC) accompany the enteric nerve plexuses that run between the successive smooth muscle layers of the gastrointestinal tract: IC-MY in the longitudinal layer as they are associated with the myenteric plexus; and IC-SM in the circular muscle layer. They form an extensive network through connections via gap junctions, and also form similar junctions with adjacent smooth muscle cells. ICC are believed to be pacemaker cells in the gastrointestinal tract as evidenced by spontaneous electrical signals arising from ICC and propagating to smooth muscle cells [402-405]. ICCs are probably the target for neuroeffector nerves from the autonomic and enteric systems supplying the gastrointestinal tract, rather than the smooth muscle cells themselves [406]. Rhythmical electrical activities in ICCs varies in cells from different sites in the gastrointestinal tract; however most are mediated by transient increases of the intracellular [Ca^{2+}] arising from intracellular stores that subsequently open Ca^{2+}-dependent ion channels to generate transient depolarisations [407]. The particular roles of intracellular Ca-stores, including the endoplasmic reticulum and mitochondria, vary in cells from different sites. ICCs express a surface marker to a receptor tyrosine kinase, *kit*, that not only acts as a characterising feature of the cell but is also vital for cell function.

Within the anorectum ICCs have been identified but their distribution is heterogeneous. In the rectum there were dense networks in the myenteric and submucosal layers, whereas in the anal sphincter they were confined to regions around the muscle bundles [408]. In the rectum ICCs have been shown to be the target of relaxatory nitrergic fibres, as heterozygous *kit* knock-out mice, as well as those deficient in nitric oxide synthase demonstrate impaired relaxation [409]. Others have suggested that ICC may not be responsible for all aspects of anal tone, rather that they determine intrinsic tone of the tissue. However, more complex reflexes, such as the recto-anal reflex (RAR; i.e. relaxation of the internal anal sphincter in response to anal stretch) may be more independent of ICC [410]. Furthermore, the latter study showed that different sources of NO mediated different functions – endothelial NOS regulated basal tone and neuronal NOS mediated the RAR and relaxation mediated by electrical stimulation of nitrergic fibres. Thus the precise inter-relationship between ICCs, smooth muscle cells and NOS-dependent muscular tone in the ano-rectum remains to be clarified.

5. FUTURE DIRECTIONS

There is still an incomplete knowledge of the factors

that regulate the contractile state of the smooth and skeletal muscles of the ano-rectum, as well as the role of the enteric and extrinsic nerve supplies, along with the interstitial cells that seem to mediate between nerves and smooth muscle. Much data obtained from studies of obstructive conditions can shed light on these control system and be used to understand better failure of continence mechanisms. Furthermore, the celluar biology that underlies sensation in the ano-rectum, as a basis of sampling reflexes for example, is little understood. However, several studies have shown that a subset of patients with faecal incontinence demonstrates an increase of urgency that may underlie the condition [411-413]. An interesting aspect is whether the emerging field of lower urinary tract sensation may be applied to the analogous situation in the ano-rectum.

VIII. NOVEL MOLECULAR TARGETS

1. INTRODUCTION

The previous sections have described recent advances in the multiplicity of mechanisms that regulate lower urinary tract function, and that may undergo changes associated with pathological changes to function. Various signal transduction mechanisms have been implicated in these controls that may make suitable targets for manipulation of function. According to the most recent classification [414], signal transduction mechanisms have been sub-classified into seven categories, namely.

- 7TM (seven-transmembrane helix) metabotropic receptors of the G protein-coupled superfamily;
- Transmitter-gated channels;
- Ion channels;
- Catalytic receptors;
- Nuclear receptors;
- Transporters;
- Enzymes.

This report will highlight several examples from the first and third categories as sources of novel targets for the treatments of urinary incontinence. The following terminology and definitions are derived from the most recent classification, “Guide to Receptors and Channels, 3rd Edition [414]. Emphasis in this section will be on targets covered less comprehensively above.

2. METABOTROPIC RECEPTORS

a) Acetylcholine-Muscarinic receptors

Among the many 7TM (metabotropic) receptors, muscarinic receptors are the most important for urinary bladder contraction, and currently for treatment of the overactive bladder.

The urinary bladder is profusely supplied with autonomic nerve fibers, which form a dense plexus among the detrusor smooth muscle cells. The majority of these nerves are excitatory cholinergic and contain acetyl cholinesterase [415]. Whilst they occur in profusion throughout the muscle coat of the bladder, some muscle bundles are more richly innervated than others. Thus, normal human detrusor contraction is mediated almost exclusively through muscarinic receptor stimulation by released acetylcholine, and responses are completely abolished by atropine [416]. Detrusor strips from normal human bladders produce little response to single stimuli and require repetitive activation of the intrinsic nerves to induce a response.

Molecular cloning studies have revealed five distinct genes for muscarinic ACh receptors in rats and humans, and five receptor subtypes ($m_{1\text{-}5}$) correspond to these gene products [416] – see also section V.1. Muscarinic receptors are coupled to G-proteins; M_1, M_3 and M_5 preferentially couple by G_q to phosphoinositide hydrolysis and diacylglcerol production, and M_2 and M_4 couple to G_i and inhibit adenylate cyclase activity. The abundance and roles of the various receptors have been considered above (Sections III and V).

Desensitization of muscarinic acetylcholine receptors is one mechanism that may cause detrusor smooth muscle to become less sensitive to incoming stimuli. This is mediated by phosphorylation of the muscarinic acetylcholine receptor by guanosine phosphate binding G-protein coupled receptor kinase (GRK) [417-419], and m_2 and m_3 GRK_2 mRNAs have been described. Protein expression of GRK_2 in normal bladder detrusor is significantly higher in obstructed bladder detrusor in patients with benign prostatic hyperplasia [419]. Failure of the desensitizing mechanism may therefore contribute to detrusor overactivity with bladder outlet obstruction.

Non-neuronal acetylcholine release is also described, from the bladder urothelium, or the suburothelial space, although its function in unknown (see also section II). The non-neuronal acetylcholine release is increased by stretch, is increased with age [420] and may contribute to pathogenesis of overactive bladder.

b) Adrenergic beta-receptors

During urine storage, sympathetic nerve activity to the lower urinary tract is important: with both relaxation of bladder smooth muscle, via adrenergic ß-receptors, and contraction of urethral smooth muscle via adrenergic β_1-receptors [421] - (see also section V.2). There are three α-receptor subtypes, $\beta_{1\text{-}3}$, and gene expression of β_3 receptors and relaxation of human detrusor via the same receptors has been recently reported [249,422-424]. Several β_3-agonists (KUC-7483, YM-178, FK-175) have been developed, and are undergoing clinical trials.

The beta-adrenoceptor is a G_s-protein-coupled receptor and activation elevates smooth muscle camp, which may trigger relaxation of smooth muscle. Downstream effectors activated via a cAMP-dependent mechanism(s) include plasma membrane K^+ channels, such as the large-conductance, Ca^{2+}-activated K^+ (BK_{Ca}) channel. ß-adrenoceptor-mediated relaxant mechanisms also include cAMP-independent signaling pathways, suggested by several pharmacological and electrophysiological studies. In airway smooth muscle, direct activation of the BK_{Ca} channel by G_s-α is a mechanism by which stimulation of $ß_2$-adrenoceptors elicits muscle relaxation independently of the elevation of cAMP [425].

The α_3 adrenoreceptor is recognized as an attractive target for drug discovery, as activation of $ß_1$ or $ß_2$-receptors can have undesirable side effects such as tachycardia or muscle tremors. Consequently, recent efforts have been directed toward the design of selective $ß_3$ agonists [145]. GW427353, a novel agonist, evokes bladder relaxation and facilitates bladder storage mechanisms in the dog [146]. The ?$_3$ agonist CL-316243 increases urine storage in SHRs [147].

c) Endothelin

The three isoforms of the 21-amino-acid peptide endothelin (ET-1, ENSG00000078401), ET-2 (ENSG00000127129) and ET-3 (ENSG00000124205) mediate their actions via the 7TM receptors ET_A and ET_B [426]. Non-selective peptide (e.g. TAK044, pA_2 8.4) and non-peptide (e.g. bosentan, pA_2 6.0–7.2; SB209670, pA_2 9.4) antagonists can block both ET_A and ET_B receptors. The predominant role of ET_A receptors in the contractile effects of ETs in the detrusor has been confirmed by several other investigators in animal, as well as in human bladders [427,428]. Features of ETA and ETB receptors are summarised in **Table 1**.

In human detrusor, ET-1-induced contractions are mediated mainly by the ET_A receptor and not by the ET_B receptor. RT-PCR revealed positive amplification of the ET_A, but not ET_B, receptor mRNA fragments [429]. This is in contrast to findings in guinea pig bladder, where both ET_A and ET_B receptors contributed to ET-induced contraction [430]. The functional role of ETs in the detrusor has not been established. The slow onset of the contractile effects seems to preclude direct participation in bladder emptying. It has been suggested that ETs may be involved in regulation of detrusor muscle tone by a direct effect [430]. However in the rat bladder ET-1 potentiates the contractions evoked by both transmural nerve stimulation and applications of ATP at peptide concentrations 10-fold below those needed to produce an increase in bladder tone [427]. This suggests a modulatory effect on detrusor neurotransmission. The selective ET-A antagonist LU 302146 acts on the atropine-resistant component of efferent detrusor activation since additional administration of atropine almost completely abolished detrusor contraction. This observation raises the possibility that ET-receptor antagonists might be beneficial in patients with neurogenic bladder dysfunction or in patients with functional or anatomical BOO [431].

Table 1. Endothelin receptors. The values in parenthesis for the selective antagonists are pKi values

Nomenclature	**ET_A**	**ET_B**
Ensembl ID	ENSG00000151617	ENSG00000136160
Principal transduction	$G_q/_{11}$, G_s	$G_q/_{11}$, $G_{i/o}$
Potency order	ET-1, ET-2>ET-3	ET-1, ET-2, ET-3
Selective agonists	—	[Ala1,3,11,15]ET-1 sarafotoxin S6c IRL1620 BQ3020
Selective antagonists	A127722 (9.2–10.5) LU135252 (8.9) SB234551 (8.7–9.0) PD156707 (8.2–8.5) FR139317 (7.3–7.9, BQ123 (6.9–7.4) BQ788 (8.4)	A192621 (8.1) IRL2500 (7.2), Ro468443 (7.1)
Probes	[3H]-S0139 (0.6 nM) [3H]-BQ123 (3.2 nM [125I]-PD164333 (0.2 nM) [125I]-PD151242 (0.5 nM)	[125I]-IRL1620 (20 pM) [125I]-BQ3020 (0.1 nM) [125I]-[Ala1,3,11,15]ET-1 (0.2 nM)

ET-1 and ET_A receptors might be involved in the generation of premicturition contractions in BOO rats, and endothelin ET_A receptor antagonists, such as YM598, may have ameliorating effects in patients with bladder overactivity associated with BOO [432]. ET_A receptor inhibition could be an effective treatment for neurogenic bladder overactivity in pathological conditions such as SCI [433]. ET_A receptors play an important role in the lower urinary tract contraction, and that the selective endothelin ET_A receptor antagonist YM598 has ameliorating effects on various urinary dysfunctions, including benign prostatic hyperplasia [434].

d) $GABA_B$

Functional $GABA_B$ receptors are formed from the heterodimerization of two similar 7TM subunits termed $GABA_{B1}$ ENSG00000168760; $GABA_{B2}$ ENSG00000136928. Principal transduction is via Gi/o. Selective agonists are 3-APPA, 3-APMPA (CGP 35024,), (*R*)-(-)-baclofen, and CGP44532, and selective antagonists are CGP62349, CGP55845, SCH50911, 2-hydroxy-s-(-)-saclofen and CGP35348.

Detrusor overactivity may be controlled by modulating the afferent input from the bladder and the excitability of the sacral reflex centre and suggests a novel method to treat overactive bladder patients with oral gabapentin [435]. Fourteen of 31 patients with refractory OAB and nocturia improved with oral gabapentin. Gabapentin was generally well tolerated and can be considered in selective patients when conventional modalities have failed [436].

e) Glutamate metabotropic receptors

There are two major classes of glutamate receptors, the ionotropic receptors which form ligand-gated cation channels [437] and the metabotropic receptors (mGluRs) which are a family of G-protein coupled receptors activating distinct signal transduction pathways in neurons [438]. The former includes *n*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-lisoxazole-4-propionic acid (AMPA), and kainite receptors, which have been revealed to play essential roles in the control of micturition reflexes [439,440]. The latter, mGluRs are constituted of eight subtypes ($mGluR_{1-8}$) which are placed into three groups on the basis of sequence homology, transduction mechanism and agonist pharmacology, and less is yet known about the functional roles in the lower urinary tract. These studies examined whether the group I mGluRs (ie, mGluR1 and mGluR5) participated in the micturition reflex of decerebrate, unanesthetised mice. Glutamate is involved in many CNS functions, and drugs acting on the different glutamate receptors may affect not only micturition [441, 442].

Female wild-type C57BL/6 mice and mGluR1 knockout mice under decerebrate, unanesthetised conditions were used for in vivo cystometry with 6-methyl-2-(phenylethynyl)pyridine (MPEP, 0.3-30 mg/kg i.p.), a selective $mGluR_5$ antagonist. Inter-micturition interval (IMI) was measured during continuous infusion cystometrograms. Blocking $mGluR_1$, $mGluR_5$ or both increased bladder capacity and $mGluR_1$ and $mGluR_5$ additively interacted to transmit afferent signals from the bladder (**figure 22**). Thus, a group I mGluR antagonist, which blocks both $mGluR_1$ and $mGluR_5$, would exert a beneficial effect more potently than a drug targeted at either $mGluR_1$ or $mGluR_5$ alone. Therefore, these may be promising drugs to treat storage dysfunctions, including detrusor overactivity and urgency urinary incontinence [443].

f) Prostanoid receptors

Prostanoid receptors are activated by the endogenous ligands prostaglandin (PG) D_2 (D), PGE_2 (E), $PGF_{2\alpha}$ (F), PGH_2 (H), prostacyclin [PGI2 (I)] and thromboxane A_2 (TX). Measurement of the potency of PGI_2 and TXA_2 is hampered by their instability in physiological salt solution; they are often replaced by cicaprost and U46619, respectively, in receptor characterization studies. Prostanoid actions are mediated by specific receptors on cell membranes, including DP, EP, FP, IP, and TP receptors that preferentially respond to PGD_2, PGE_2, PGF_2, PGI_2, and TXA_2, respectively. The EP receptor itself is subdivided into four subtypes: EP_1, EP_2, EP_3 and EP_4 (**table 2**) [444, 445].

The signaling pathways vary. For example, TP receptors signal via G_q protein, activating IP_3/diacylglycerol pathways, but also other G-proteins may be involved. EP_1 receptors signal via IP_3 generation and increased cell Ca^{2+}; activation of EP_2 and EP_4 leads to an increase in cAMP; and EP_3 activation inhibits cAMP generation via a pertussis toxin-sensitive G_i-coupled mechanism and may also signal via the small G-protein, rho. Prostanoids may affect excitation-contraction coupling in the detrusor in two ways, directly by effects on the smooth muscle, and/or indirectly via effects on neurotransmission.

The prostanoid receptor most important for detrusor function has not been established. Mice lacking EP_1 receptors had normal cystometry, but did not react to intravesical PGE_2 instillation, which caused detrusor overactivity in wild-type controls. Obstruction of EP_1 receptor-knockout mice did not prevent the resulting increase in bladder weight but prevented the increase in spontaneous contractile activity (non-voiding contractions) seen in wild-type controls [231]. Prostaglandin E_2 enhances the micturition reflex through C-fiber afferents via EP_1. Therefore, EP_1 selective antagonists may improve bladder storage function [446]. However, EP receptor distribution and the implications for bladder mucosa function are not fully understood. EP_2 and EP_4 mRNA are over-expressed in the urothelium of obstructed human urinary bladder compared to non-obstructed bladder.

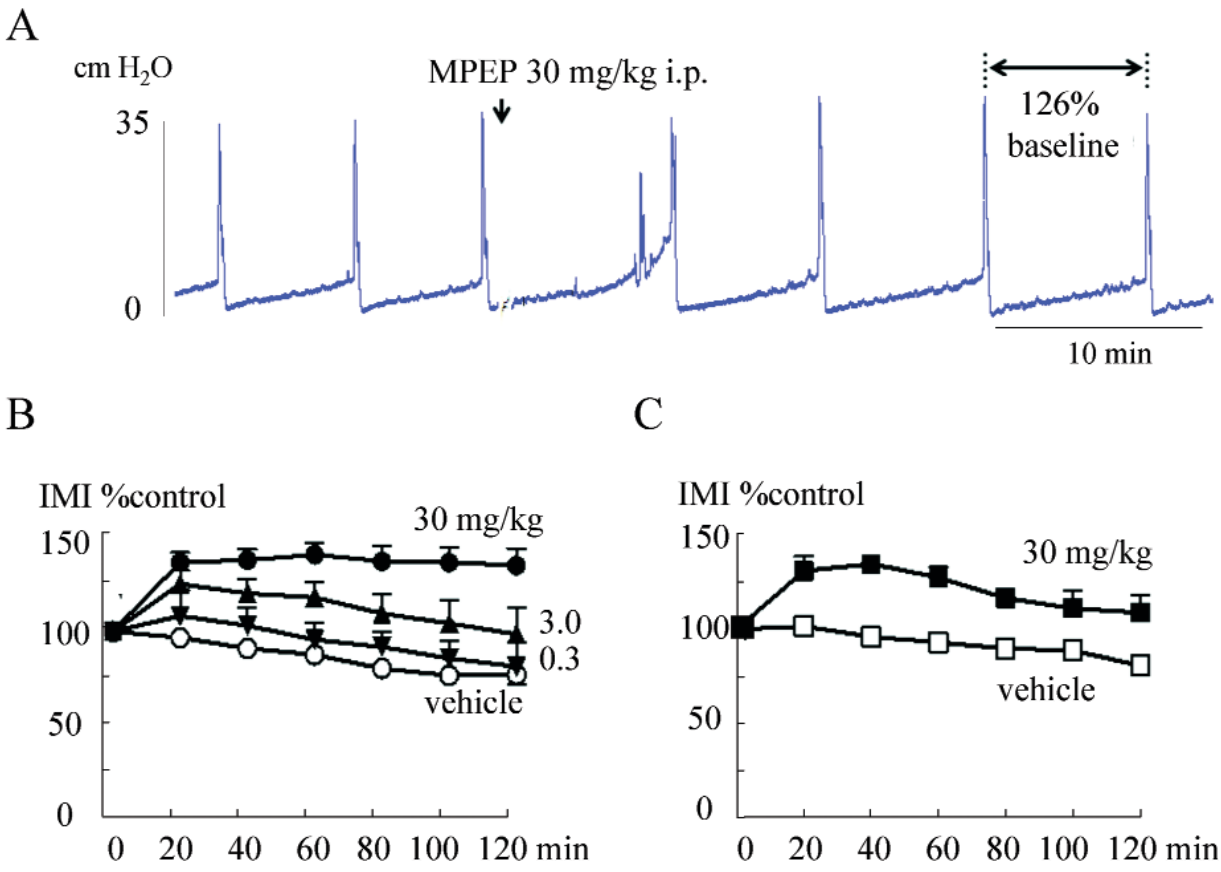

Figure 22 : Modulation of mGlu receptor activity and continuous infusion cystometry. A: effect of 6-methyl-2-(phenylethynyl)pyridine (MPEP, 30 mg/kg i.p.), a selective mGluR5 antagonist, on bladder activity during continuous infusion (30 µl/min) in a decerebrate, unanesthetized mouse. The dose increased the inter-micturition interval approximately 26 % of the baseline value in this animal without a suppression of micturition pressure. B: the time-course of the effect of MPEP (0.3, 3 and 30 mg/kg i.p.) or vehicle on inter-micturition interval (IMI) in decerebrate, unanesthetized wild type mice. C: the time-course of the effect of MPEP (30 mg/kg i.p.) or vehicle on IMI in decerebrate, unanesthetized mGluR1 knockout mice. The peak increase of IMI was similar to that in wild-types. M Takeda, unpublished data

Table 2. Prostanoid receptors. The values in parenthesis for the selective antagonists are pKi values

Nomenclature	**EP1**	**EP2**	**EP3**	**EP4**
Ensembl ID	ENSG00000 160951	ENSG00000 125384	ENSG00000 050628	ENSG00000 171522
Principal transduction	Gq/11	Gs	Gi/o	Gs
Rank order of potency	E>F,I>D,T	E>F,I>D,T	E>F,I>D,T	E>F,I>D,T
Selective agonists	17-Ph-ϖ-trinor-PGE2, ONO-DI-004	Butaprost, AH13205, ONO-AE1–259	Sulprostone, SC46275, ONO-AE1–329	ONO-AE-248
Selective antagonists	ONO8711 (9.2), SC51322 (8.8)	-	L798106 (7.7)	GW627368 (9.2), ONO-AE3–208 (8.5), L161982 (7.6)
Probes	[3H]-PGE2 (1–25 nM)	[3H]-PGE2 (5–22 nM)	[3H]-PGE2 (0.3–7 nM)	[3H]-PGE2 (0.6–24 nM)

This overexpression significantly correlated with International Prostate Symptom Scores (IPSS), especially the storage IPSS component [447]. Hence, in contrast to the previous mouse data, EP_2 and EP_4 could be promising receptor subtypes for the treatment of overactive bladder [446].

3. ION-CHANNELS

a) Calcium (voltage-gated)-channels

Calcium (Ca^{2+}) channels are voltage-gated ion channels present in the membrane of most excitable cells and form hetero-oligomeric complexes. The α_1 subunit is pore-forming and provides the extracellular binding sites for practically all agonists and antagonists. The ten cloned α–subunits can be grouped into three families:

- a) the high-voltage activated dihydropyridine-sensitive (L-type, CaV1.x) channels;
- b) the high-voltage activated dihydropyridine-insensitive ($Ca_V2.x$) channels and
- c) the low-voltage-activated (T-type, $Ca_V3.x$) channels.

Each α_1 subunit has four homologous repeats (I–IV), each repeat having six transmembrane domains and a pore-forming region between transmembrane domains S5 and S6. Gating is thought to be associated with the membrane-spanning S4 segment, which contains highly conserved positive charges. Many of the α_1-subunit genes give rise to alternatively spliced products. At least for high-voltage activated channels, it is likely that native channels comprise co-assemblies of α_1, β and $\alpha_{2\text{-}\delta}$ subunits. The γ subunits have not been proven to associate with channels other than α_{1s}. The $\alpha_{2\text{-}\delta1}$ and $\alpha_{2\text{-}\delta2}$ subunits bind gabapentin and pregabalin.

Activation of detrusor muscle, both through muscarinic receptor and NANC pathways, seems to require both influx of extracellular Ca^{2+} through Ca^{2+} channels and mobilization of intracellular Ca^{2+} [448,449]. The importance of each mechanism may vary between species and also with respect to the transmitter studied.

b) Epithelial Na^+ channels (ENaC)

Epithelial Na^+ channels (ENaC) are responsible for sodium reabsorption by the epithelium lining the distal part of the kidney tubule, and fulfil similar functional roles in some other tissues such as the alveolar epithelium and the distal colon. This reabsorption of Na^+ is regulated by aldosterone, vasopressin and glucocorticoids, and is one of the essential mechanisms in the regulation of sodium balance, blood volume and blood pressure.

The degenerin epithelial Na^+ channel (ENaC) family has been proposed as a transducer of sensory stimuli in several species [450-453]. These ENaCs seem to be mechanosensitive (MS) according to many findings. In the rabbit urinary bladder, ENaC changes its Na^+ transporter properties after changes of hydrostatic pressure [451]. ENaC in the renal pelvic epithelium of rats participates in the activation of afferent renal MS neurons by increased renal pelvic pressure [31]. Thus, ENaCs are likely to be involved in MS transduction in the bladder, and may be related to the pathophysiology of changes in sensory nerve function by upregulation of ENaCs in the bladder epithelial cells or afferent nerve terminal. ENaC is expressed in the mammalian urinary bladder and it has been proposed that amiloride-sensitive Na^+ transport across the apical membrane of the mammalian urinary bladder epithelium is mediated primarily by ENaC [454].

1. ENaC in human urinary bladder:

The α-, β-, and γ-subunit ENaC proteins and mRNA are clearly expressed in human bladder epithelium with and without BOO. The quantified ENaC mRNA expression correlates significantly with the storage symptom score (figure 4).

2. ENaC in rat and rabbit urinary bladder:

Intravesical infusion of amiloride (1 mM) significantly reduces the frequency of reflex voiding during bladder filling, increases bladder capacity, with no effect on the amplitude of micturition pressure. Stretch (50%) induced significant increase in ATP release from whole layer bladder strips, but only a slight increase in muscular layer strips without epithelium. Amiloride (1 mM) significantly suppressed stretch-evoked ATP release from bladder epithelium [455,456]. cAMP stimulates the insertion of new Na^+ channels into the apical membrane of the rabbit bladder epithelium [457].

c) Potassium channels: The 6TM family: Maxi-K channel

Potassium channels are fundamental regulators of excitability. They control the cell membrane potential, the frequency and the shape of the action potential, and the secretion of hormones and neurotransmitters. Their activity may be regulated by transmembrane voltage, Ca^{2+} and neurotransmitters (and the signalling pathways they stimulate). They consist of a primary pore-forming α-subunit often associated with auxiliary regulatory subunits. The three main families are the 2TM (two transmembrane domain), 4TM and 6TM families.

The 6TM family of K channels comprises the voltage-gated K_V subfamilies, the KCNQ subfamily, the EAG subfamily (which includes HERG channels), the Ca^{2+}-activated *slo* subfamily (actually with 7TM) and the Ca^{2+}-activated SK subfamily. As for the 2TM family, the pore-forming α-subunits form tetramers and heteromeric channels may be formed within subfamilies (e.g. $K_V1.1$ with $K_V1.2$; KCNQ2 with KCNQ3).

Large-conductance, voltage- and Ca^{2+}-activated K^+ (Maxi K, or BK_{Ca}) channels regulate the resting potential and repolarization of the action potential, and play a critical role in modulating contractile tone of smooth muscle, including the detrusor [107,458], and neuronal processes. Not only BK_{Ca} channels, but also small-conductance (SK_{Ca}) channels, are regulators of excitability in detrusor smooth muscle. Ca^{2+} entry through voltage-dependent Ca^{2+} channels activates both BK_{Ca} and SK_{Ca} channels, but Ca^{2+} release (Ca^{2+} sparks) through ryanodine receptors activates only BK_{Ca} channels [459, 460]. Thus, BK_{Ca} channels are more important in counteracting enhanced spontaneous mechanical activity with urinary bladder smooth muscle stretch. Phasic contractions of human detrusor, due to transmitter release, are dependent on calcium entry through L-type Ca^{2+} channels and BK_{Ca} channels have a very significant role in reducing both cholinergic- and purinergic-induced contractility [112]. Thus, Ca^{2+}-dependent K^+ channels could contribute to pathologies such as overactive detrusor and represent attractive pharmacological targets for decreasing phasic contractions of detrusor smooth muscle in OAB [461]. The selective antagonist for BK_{ca} channels, NS-8, decreased the discharge rate of the afferent pelvic nerve on bladder filling in rats [122]. Thus, NS-8 might have the potential for treating patients with urinary frequency and incontinence.

The importance of the BK_{Ca} channel is also demonstrated by the observation that local h-*slo* cDNA (i.e., the BK_{Ca} channel) injection ameliorated detrusor overactivity in a rat model of partial urinary outlet obstruction [113].

It was proposed that expression of h-*slo* in rat bladder functionally antagonized the increased contractility normally observed in obstructed animals, and thereby improved detrusor overactivity and also indicated a potential utility of gene therapy for urinary incontinence. Consistent with increased urinary bladder contractility caused by the absence of BK_{Ca} currents, *slo*(-/-) mice demonstrate a marked elevation in urinary frequency [462].

BK_{Ca} channels consist of distinct α and β-subunits. Whereas only one α-subunit has been identified until now, four putative β-subunit types have been cloned. The existence of BK_{Ca} α- and β_1-subunits in human urinary bladder (both in the mucosal layer and the detrusor) have been demonstrated with a combination of genetic and immunohistochemical methods.

The expression level of BK_{Ca} channels in the detrusor muscle and the mucosal layer decreased in BOO bladders compared with controls, consistent with the possibility that they have a pivotal role in the generation of OAB induced by bladder outlet obstruction [463] (**figure 23**).

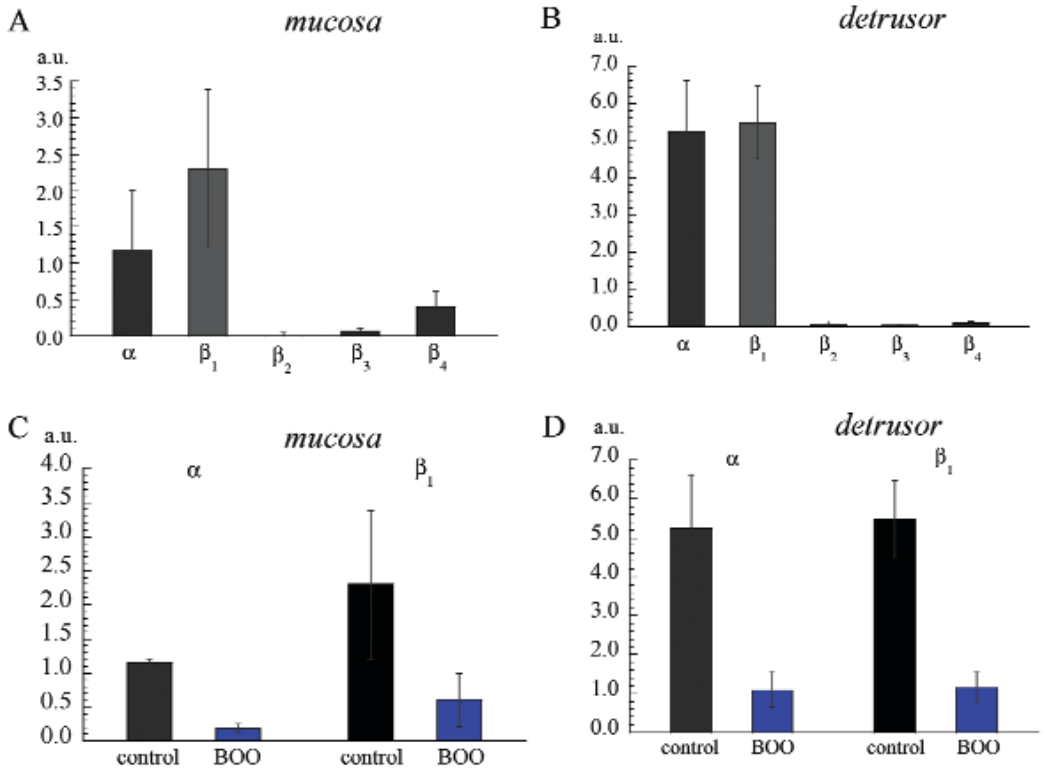

Figure 23: Quantitative RT-PCR of each subunit of the BK channel. A,B: Quantitative RT-PCR in mucosa and detrusor, the a- and b1-subunit genes were predominant. C,D: Comparison of α- and ß1-subunit gene expression in mucosa and detrusor from normal and obstructed bladders; obstruction deceased expression of both subunits, in both layers. Amounts are expressed in arbitrary units relative to a housekeeping gene. Modified from [454].

A novel BK_{Ca} channel blocker, A-272651, represents one of the first small molecules that could be used to characterise BK_{Ca} channels in physiological and pathological states [464]. No other K^+ channel opener seems to have passed the proof of concept stage, and there is at present no convincing evidence showing that K^+ channel opening is a useful principle for treatment of detrusor overactivity [465] The safety and tolerability of escalating doses of hMaxi-K, a gene transfer product of the human Maxi-K (BK_{Ca}) channel, were confirmed by clinical and laboratory tests in 11 patients with moderate to severe erectile dysfunction. hMaxi-K gene transfer is potentially a viable approach to the treatment of erectile dysfunction and other smooth muscle diseases with targeted access [466].

d) Transient receptor potential (TRP) cation channels

The TRP superfamily of cation channels, whose founder member is the *Drosophila* Trp channel, can be divided, in mammals, into six families; TRPC, TRPM, TRPV, TRPA, TRPP and TRPML based on amino acid homologies. TRP subunits contain six putative transmembrane domains and assemble as homo- or hetero-tetramers to form cation selective channels with varied permeation properties. The TRPC ('Canonical') and TRPM ('Melastatin') subfamilies consist of seven and eight different channels, respectively (*i.e.*, $TRPC_{1-7}$ and $TRPM_{1-8}$). The TRPV ('Vanilloid') subfamily comprises six members ($TRPV_{1-6}$) whereas the TRPA (Ankyrin) subfamily has only one mammalian member ($TRPA_1$). The TRPP ('Polycystin') and TRPML ('Mucolipin') families are not fully characterised. Established, or potential, physiological functions of the individual members of the TRP families are discussed in detail in the recommended reviews and are only briefly mentioned here [467,468].

The importance of stretch-evoked ATP release from urothelium, and the subsequent activation of afferents has been described above (section II). The nature of the mechnosensitive (MS) processes responsible for ATP release is unclear but ENaCs and TRP ion channels are candidates [469]. With respect to TRP channels several have been identified, and many also have thermosensing properties [470].

Among several thermosensing TRP channels, $TRPA_1$, $TRPM_8$, $TRPV_1$, and $TRPV_4$ are candidates of molecular targets of the novel treatments for urinary incontinence (**table 3**) [471]. $TRPA_1$ may be a candidate for an MS channel, with cold-sensing capability [472,473]. In addition to activation by mechanical stimuli, $TRPA_1$ is also a cold receptor: activation is initiated when the temperature is decreased to 17ºC [474]. Intravesical infusion of ice water elicits uninhibited contraction of bladder in infants and patients with neurogenic bladder and it is reasonable to assume that $TRPA_1$ is involved in the bladder-cooling reflex and should provide a further treatment target [475].

• TRPA1 and $TRPM_8$ in the rat and the human urinary bladder:

Whole mount double-staining demonstrates expression of $TRPA_1$ on the CGRP-reactive sensory nerve termini, but not on mucosa of the bladder (**figure 24**) and is much greater in the obstructed bladder. Expression of mRNA and protein of $TRPA_1$ and $TRPM_8$ were confirmed by quantitative RT-PCR and immunohistochemstry. During cystometry, 0.6 mM *trans*-cinnamaldehyde (CA; an agonist of $TRPA_1$) decreased the pressure threshold (PT), inter-micturition interval (IMI) and micturition pressure (**figure 25**) – similar results were obtained with AITC: allyl-isothiocyanate. The effects on PT and IMI were completely reversible. Desensitization of C-fibres by capsaicin significantly attenuated the effects of 0.6 mM CA on PT and IMI (p=0.022, 0.002). These results, couple to the fact that $TRPA_1$ expression is increased in the obstructed bladder, suggest that both $TRPA_1$ and $TRPM_8$ may contribute to the pathophysiology of OAB [476,477].

e) ER stress

In bladder outlet obstruction (BOO), mechanical stress and ischemia/hypoxia are implicated in structural and functional alterations to the urinary bladder. In other organs, mechanical stress and hypoxia trigger a

Table 3. Possible implication between TRP ion channels and lower urinary tract function

Channels	Agonist	Thermo-threshold	Sense	Localization
TRPA1	Isothio-cyanate (Mustard)	<17°C	Pain, Hot Cold ?	Urinary bladder
TRPM8	Menthol	<28°C	Cool, Menthol	Urinary bladder, Prostate
TRPV1 (vanilloid receptor)	Capsaisin (hot pepper)	>43°C	Pain, Hot, Burning	Urinary bladder, Prostate?
TRPV4		>28°C	Warm ?	Urinary bladder

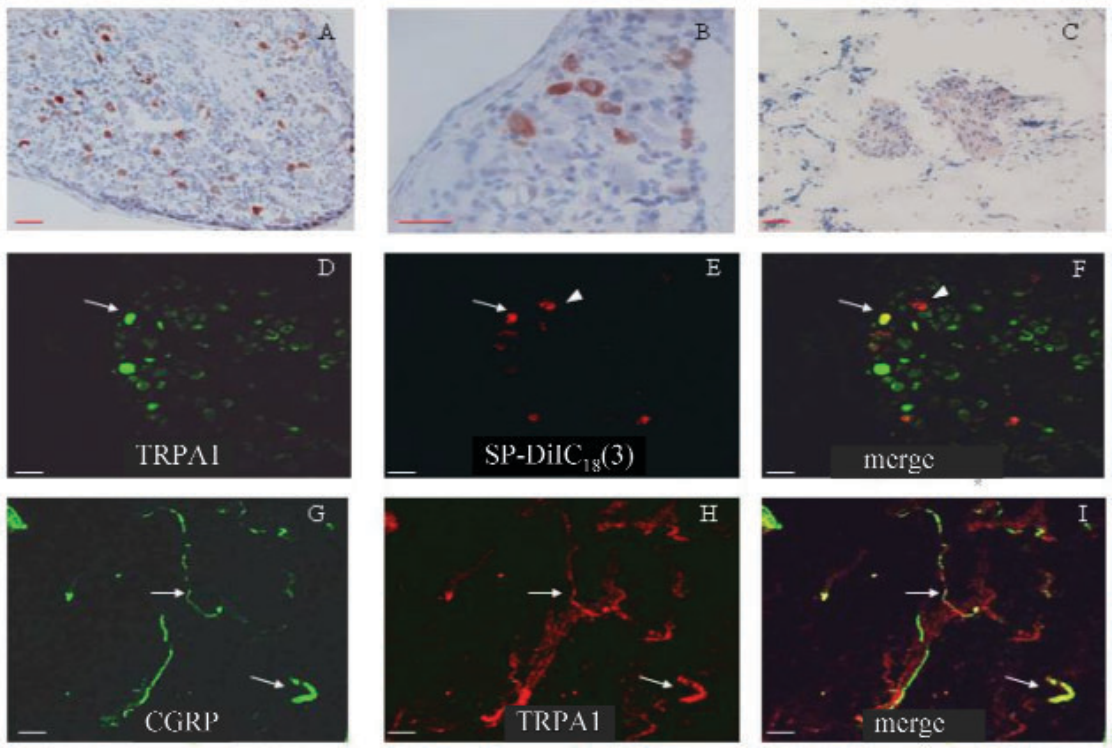

Figure 24 : Expression and localization of TRPA1 protein in L6-S2 dorsal root ganglia (DRG) and smooth muscle of the rat urinary bladder. A,B: TRPA1 immunoreactivity at small to medium sized neurons of L6-S2 DRG. C: TRPA1 immunoreactivity in the detrusor smooth muscle layer. D-F: The DRG neurons innervating the bladder were labelled retrogradely by injecting SP-DiIC$_{18}$(3) into the urinary bladder wall two weeks earlier. Some SP-DiIC$_{18}$ positive neurons expressed TRPA1 (arrow) while some others not (arrow head). This is most clearly seen in the merged picture (yellow). G-I: TRPA1 also co-localizes with CGRP at the nerve termini in the lamina propria of urinary bladder (arrow). Scale bar: 50 _m. M Takeda, unpublished data

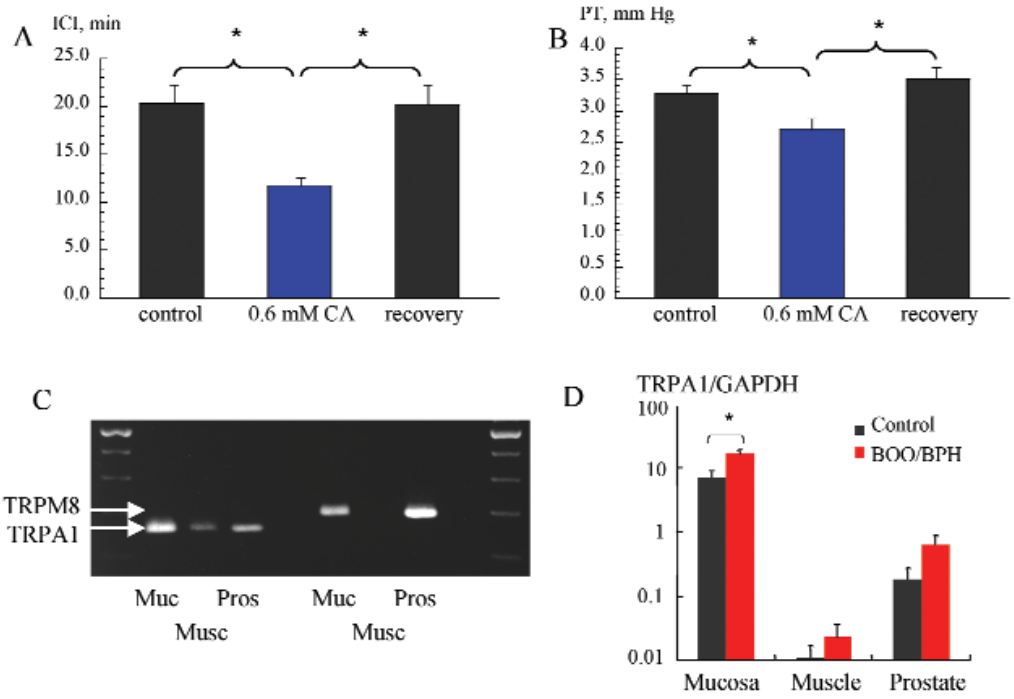

*Figure 25: TRPA1 and TRPM8 function and expression. A,B: in vivo rat cystometry using physiological saline solution or TRPA1 agonist (CA: trans-cinnamaldehyde) under urethane anesthesia. A; the effect of 0.6 mM cinnamaldehyde (CA) on inter-contraction interval (ICI), *p=0.05. B; the effect of 0.6 mM cinnamaldehyde (CA) on pressure threshold (PT). C: the expression of TRPA1 and TRPM8 mRNA in the human bladder mucosa (muc), detrusor muscle (musc) and prostate (pros). D: the expression of TRPA1 mRNA was significantly higher in the mucosa of BOO (bladder outlet obstruction) than non-obstructed human bladder, but not in the muscle layer, nor in the prostate, p<0.01. M Takeda, unpublished data*

particular response, the endoplasmic reticulum (ER) stress response. ER stress is defined as accumulation of unfolded or misfolded proteins in the ER, which induces a coordinated adaptive program called unfolded protein response (UPR). UPR alleviates ER stress by suppression of protein synthesis, facilitation of protein folding via induction of ER chaperones, including a 78 kDa glucose-regulated protein (GRP78) and reinforced degradation of unfolded proteins. If the stress is beyond capacity of the adaptive machinery, however, cells undergo apoptosis via several mechanisms including induction of CCAAT/enhancer-binding protein-homologous protein (CHOP) [478].

Hence, the involvement of ER stress in the damage of the bladder caused by BOO has been examined. An experimental model of BOO was established in rats by complete ligature of the urethra for 24 h, and bladders were subjected to Northern blot analysis and assessment of apoptosis. Isolated urinary bladders and bladder-derived smooth muscle cells (BSMCs) were also exposed to mechanical strain and hypoxia and used for analyses. To examine the involvement of ER stress in bladder damage, effects of a chemical chaperone, 4-phenylbutyrate (4-PBA), were evaluated *in vitro* and *in vivo*. Outlet obstruction for 24 hours induced expression of ER stress markers, *GRP78* and *CHOP*, in the bladder, and was associated with induction of markers for mechanical stress (cyclo-oxygenases 2) and hypoxia (vascular endothelial growth factor and glyceraldehyde-3-phosphate dehydrogenase). When isolated bladders and BSMCs were subjected to mechanical strain, induction of *GRP78* and *CHOP* was not observed. In contrast, when BSMCs were exposed to hypoxic stress caused by $CoCl_2$ or thenoyltrifluoroacetone (TTFA), substantial up-regulation of *GRP78* and *CHOP* was observed, suggesting involvement of hypoxia in the induction of ER stress (**figure 26**). In bladders subjected to BOO, the number of TUNEL-positive cells increased in the epithelial cells and BSMCs. Similarly, treatment with TTFA or $CoCl_2$ induced apoptosis of BSMCs, and 4-PBA significantly attenuated ER stress and apoptosis triggered by these agents. Furthermore, *in vivo* administration with 4-PBA significantly reduced apoptosis in the bladder subjected to BOO [479].

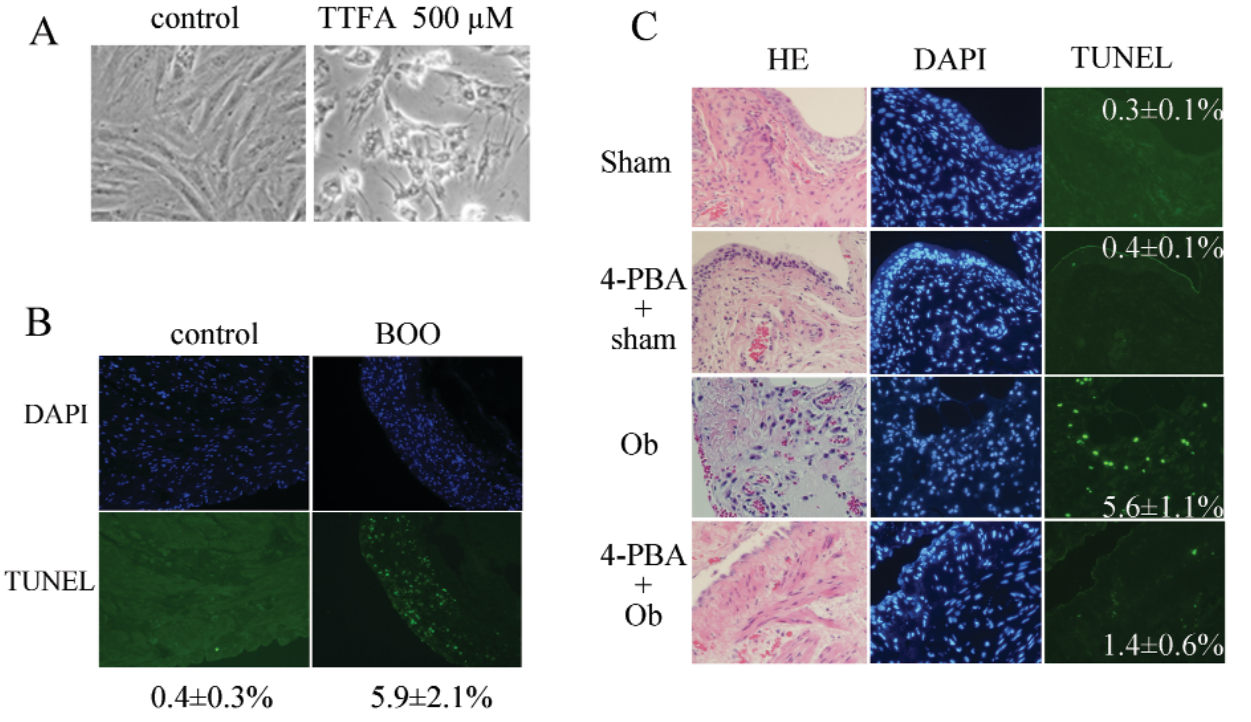

Figure 26 : Induction of apoptosis in cultured detrusor smooth muscle cells (DSMCs) by hypoxic stress and BOO and attenuation by molecular chaperones. A: Upper panels. DSMCs were exposed to 500 mM TTFA (thenoyltrifluoroacetone) for eight-hours and observed by phase-contrast microscopy. Lower panels. Induction of apoptosis in the bladder by BOO. Control bladders and bladders subjected to BOO for 24 hours were examined by DAPI staining (4',6-diamidino-2-phenylindole staining) and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labelling assay (TUNEL), to count apoptotic cells. B: Induction of apoptosis in the bladder by BOO. Control bladders and bladders subjected to BOO for 24 h were examined histologically by 4',6-diamidino-2-phenylindole staining (DAPI) and terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling assay (TUNEL). C. The effect of 4-phenylbutyrate (4-PBA) on the apoptosis of the urinary bladder indueced by BOO. Rats were administered daily with either ethanol vehicle or 4-PBA (120 mg/kg, i.p.) for three-days and subjected to BOO. Histological analyses were performed using hematoxylin-eosin (HE) and DAPI staining, and TUNEL assay. All data are means±SE (n=4). The values represent the percentage of cells undergoing apoptosis. M Takeda, unpublished data.

These results suggest that outlet obstruction caused ER stress via hypoxic stress in the bladder and that hypoxia-triggered ER stress may be involved in the induction of apoptosis. ER stress may therefore trigger BOO-induced detrusor overactivity, or OAB. Hence, chemical chaperons such as 4-PBA might be a novel pharmacotherapy for prevention of BOO-induced bladder dysfunction, or urinary incontinence (**figure 27**).

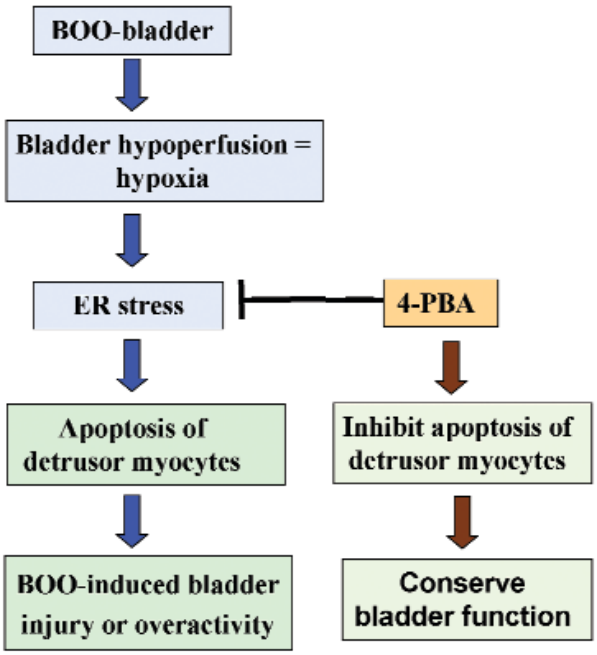

Figure 27 : Hypothesis of the possible implication of BOO, hypoxia-induced ER stress and urinary bladder injury. The possibility of novel pharmacotherapy for BOO-induced bladder injury or dysfunction by attenuation of BOO-induced apoptosis in the bladder by chemical chaperones such as 4-PBA.

IX. TISSUE REPLACEMENT AND TRANSLATIONAL RESEARCH

1. INTRODUCTION

Translational research must maintain two key components:

- A direct pathway to impacting on patient care
- An ethical responsibility to improve on current treatment

Tissue engineering is now at a stage where research is moving out of the laboratory and into the clinical arena and now demands that financial and well as scientific imperatives must be considered. A recent estimate for the development of a new tissue engineered device is in excess of €10MM, with a time span of not less than five years [480]; the NIH spent $643 million on stem cell research in 2006. The evolution of organ replacement therapy through cell culture and implantation has attracted considerable attention from both the medical profession and the media. Progress must be closely monitored to ensure that the driving forces of the commercial requirements of stakeholders balance the necessary clinical objectives.

Several groups have produced innovative and progressive work and in the last twenty years there has been a move from predictions of success to a more considered approach. The progression has been evident with an increasing understanding of the complexity of the problem. Technology has developed to allow various means of producing a pure cell culture – including better understanding of selective media, and fluorescent-activated cell sorting. An increasing number of abstracts at major urological conferences in recent years further testifies to the proximity of clinical application.

2. INJECTABLE THERAPY

This work is a step away from the production of a functional and implantable graft. Its strengths include no requirement for the use of a scaffold and the final functional outcome is largely dependant on the host's regenerative abilities.

Animal model work suggests that in the case of stem cell therapy there is cellular differentiation, and in the example of sphincteric replacement this may lead to cells with charactersitics of smooth and skeletal muscle. Some data claim a clinical cure rate apprtoaching 90% with these therapies [481], and in addition measurable parameters such as maximum urethral closing pressure can be improved with injection of stem cells [482]. Histological evidence for the generation of muscle fibres is also evident [483]. In a randomized control trial comparing the treatment against conventional injectables the use of stem cells faired far better. But there is a need for multicentre trials before such a treatment could become accepted as a standard [484].

3. TISSUE REPLACEMENT

a) Urethra

There have been attempts at urethral regeneration – most commonly by implantation of a matrix to stimulate regeneration. Acellular bladder collagen has been used to replace tissue in an animal model [485]. Success was improved by initial epidermal seeding – the cells of which disappeared six months after grafting. Growth and subsequent blood supply may be improved by adding growth factors, such as VEGF, and cell selection [486]. It appears that *ex vivo* urethral regeneration has, hitherto been unsuccessful. However, a clinical trial using tissue engineered buccal mucosa demonstrates that in a near three-year follow up series there is promise: there is a 100% initial graft

take, with two out of five patients requiring either partial or total graft excision; the remainder having patent urethras but have required instrumentation [487]. Bigger groups of patients have had small intestine submucosa (SIS) implanted as a graft for the treatment of urethral stricture disease and demonstrate a success rate of up to 85% with this technique – the poorest outcomes with reconstruction of the penile urethra [488,489]. Seeded tubular constructs have been used with success in preclinical studies and this is an area that awaits further development. However, one must compare the potential success, against conventional treatment, or even the culture of single layer grafts, and consider the resource implications of such a treatment strategy. Recent data suggest that there may be a less invasive approach to establishing urothelial cultures. Multi-layered cultures of urothelial cells were established from bladder washings from 29 patients [490]. Using careful cell selection and culture techniques it is possible that grafts could be created in this way.

b) Bladder

The complexities of generating a tissue-engineered neobladder have been widely acknowledged. In order to survive, imbibition and inosculation will not be sufficient – the graft must have an adequate vascular supply. Furthermore, for functional success the smooth muscle must be controlled i.e. with a functional innervation. The generation of a seeded co-cultured cellular construct continues to be a major research directive. Alternatives have been trialled, such as the urothelial-lined uterus [491], but have not yet reached a stage of clinical application. The issue of graft nutrition appears to be helped considerably by wrapping the graft in omentum. Such grafts appear to show reasonable *in vitro* characteristics [492], as well as improvement in some clinical parameters such as compliance and capacity [493]. The questions relating to the sensory status of the construct and the voiding function remain open. Data have shown the phenotypic similarities of cultured cells to their native counterparts [494], although this has not yet translated into in vivo success. However, a recent improvement appears with the use of dynamic cell culture [495]. Other components may be developed with the use of further stem cell technology, in conjunction with genomics.

c) Penis

A number of opportunities exist in which tissue engineering may be useful. The generation of grafts from scrotal dermis has shown promise in improvement of penile girth. In a series of 84 patients 81% rated their procedure as very good or better with a median follow-up of 24 months [496]. Possibilities for tissue replacement exists in Peyronie's disease – these include SIS, bovine pericardium and saphenous vein, alloderm and tutopatch. In a case series including 147 patients SIS and alloderm may yield consistent clinical results with success rates of 79 and 77% being quoted for functional and cosmetic outcomes, respectively [497]. Along with the structural possibilities the opportunity to restore function with cultured endothelial cells has also been tested in animal models [498]. The replacement of corporal smooth muscle may be achievable with differentiation of muscle-derived stem cells into smooth muscle cells – animal work has demonstrated possibilities for this [499].

4. APPROACHES TO GRAFT GENERATION

The components of a final graft will vary due to requirements, as illustrated by the examples above. The two key components are the scaffold and the cellular content. The scaffold in turn can be considered as 3 components:

- Support mechanism for cells
- Nutrient supply for cells
- Functional control of cells

a) Support mechanism for cells

Many potential scaffolds have been trialled. Options include the creation of a layer of urothelium; which is then seeded as a new lining and incorporated as part of another organ in augmentation. Examples have included bowel and uterine covered segments used to expand bladders [490]. It is likely that different organs and, perhaps different disease states, will require different scaffolds. With developing technology and understanding of the interaction of the scaffold and cells there is a necessity to explore this area. Improved biocompatible (CO alkene) polymers have increased cellular activity [500]. Mechanical properties have not been widely investigated. However, the growth of cells may be influenced by the physical properties of a scaffold, more than previously realised, and factors such as the elastic modulus may be important in generating optimal cell growth [501]. Cells cultured on a dynamic bioreactor, that stretched and relaxed the culture plate, improved the contractility of the resultant cells. Those cultured on a static plate showed no measurable contractility whilst preconditioned cells showed tetanic and twitch responses between 1 and 4 weeks of culture [495]. In addition work is constantly aiming to find alternative scaffolds including the amnion (although this has not been tested for urological application) as alternatives to decellularised bladder matrix and SIS [502].

b) Nutrient supply for cells

The culture of cells *ex vivo* has led to a number of solutions for providing cell nutrition. Bioreactor technology has become an area of subspecialist development in its own right. It appears that problems arise with removal from the bioreactor and implantation, and the difficulties in establishing and *in vivo* nutrient supply. Baumert *et al* have pursued the possibility of early transition of the construct to an 'omental bioreactor' for neo-ureters – with seeded

constructs being placed intra-abdominally and wrapped in omentum. Animal data suggest that this may be a successful strategy for final culturing - with the generation of a vacularised multilayered, terminally differentiated graft [492,503]. Other factors, such as pretreatment of scaffolds with growth factors, may also have both a direct effect on nutrient availability and utilisation leading to enhanced cell growth [486,504]. The possibility of growth factors stimulating specific co-culture of a capillary network have also to be considered [505].

c) Functional control of cells

There are two aspects to this: firstly, the differentiation of cells. Factors that may influence this have been mentioned but included either stimulation of cells (such as stretch for muscle cells [495]), or the use of selective media. The generation of the correct cell line is vital and forms part of the functional control. Secondly, the generation of a mechanism that will allow functional control of the final graft. The ideal will be to include sensory responses from the graft – a tissue engineered substitution cystoplasty would be completely insensate, making control difficult except by timed voiding. The motor control of an implant is also important. This does not appear to be an issue for the injectable therapies discussed earlier but may be for free grafts. Histology from animal work with such implants suggests that there is a degree of neural ingrowth at the periphery of grafts when harvested [506], however there is no specific reference to the voiding function of these grafts. Indeed the continuation of this work suggests that contractile function may only be seen in cells that have been cultured in a dynamic environment [494] – with very poor contraction seen in cells not treated in this way.

5. CONCLUSION

Current work in this field is impressive and fast moving. It is now evident that with the involvement of commercial interest the huge financial investment will be expected to generate return. The focus of this work must remain our consumer – the patient. Outcomes must be at least as good as previously available treatments in order to gain recognition and credibility and no commercial pressure should be allowed to compromise this. The complexities and interaction of different graft components have highlighted many difficulties to progress. The increased application of stem cell work and an understanding of host-regenerative strategies may overcome some of these obstacles. Dynamic culture and the further development of bioreactor technology also hold hope for further development.

It is clear that none of these therapies could be advocated outside a trial setting and that data regarding each patient needs to be carefully followed up and preserved in the interests of long term results.

X. RECOMMENDATIONS FOR LOWER URINARY TRACT AND LOWER GASTRO-INTESTINAL TRACT RESEARCH

1. Integrate data from reductionist experiments to formulate better systems-based approaches in the investigation of the pathology of the LUT and LGIT.

2. Generate improved experimental approaches to investigate the pathophysiology of the LUT and LGIT by:

- the development of characterised animals models
- use of human tissue from well-characterised patient groups.

3. Encourage greater emphasis on basic research into our understanding of tissues receiving relatively little attention: i.e. the lower gastrointestinal tract; the bladder neck and urethra.

4. Generate a more multidisciplinary approach to investigate the function of the lower urinary tract through collaborations between biological, physical and mathematical sciences.

5. Increase interaction between higher education institutions (HEIs), industry and medical centres to encourage translational approaches to research.

6. Bring about a greater emphasis on the importance of research to medical trainees through:

- establishing research training as a core component of medical training
- increased access to support funds, especially scholarships and personal awards
- organisation of focussed multidisciplinary research meetings, either stand-alone or as part of larger conferences
- greater interaction between medical centres and HEIs

7. Increase emphasis on research into lower urinary tract and gastro-intestinal tract in HEIs through:

- greater representation on grant-funding agencies
- encouragement of submission to high impact-factor journals and recognition of research published in specialty journals
- more integrated teaching and training opportunities

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